GROWTH AND FLORAL DEVELOPMENT OF FIVE SPECIES OF RANGE GRASSES IN CENTRAL OKLAHOMA[1]

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Introduction

In recent years much interest has been shown in the responses of various species of range grasses to photoperiod and other environmental factors (**1, 3, 7, 8, 10, 11, 17**). The cited studies and others were done under partially controlled experimental conditions. Therefore, it was decided to determine the natural daylengths in which inflorescences are initiated and those in which flowering occurs in five abundant species of perennial grasses under prairie conditions in central Oklahoma and to compare these results with published reports so far as possible. In addition, the time-lapses between the initiation of the inflorescence and the beginning of its exsertion and between the latter and anthesis were determined for individuals of each species investigated—side-oats grama (*Bouteloua curtipendula* [Michx.] Torr.), Indian grass (*Sorghastrum nutans* [L.] Nash), switch grass (*Panicum virgatum* L.), little bluestem (*Andropogon scoparius* Michx.), and big bluestem (*Andropogon furcatus* Muhl.).

There have also been several significant contributions concerning the relationship of developmental physiology to developmental morphology in range and cultivated cereal grasses (**5, 6, 9, 13, 14, 15, 16**). SHARMAN (**15**) stated that "the abruptness with which vegetative growth changes over to inflorescence production [in the Gramineae] suggests that there is a sort of minimum condition in the internal economy of the shoot apex which must be reached, and that once the point of balance is passed the vegetative type of growth ceases." It was at one time thought that the C/N balance might be the deciding factor. PURVIS (**13**) showed, however, that the amount of nitrogen supplied had little effect on the pattern of development of seedlings of winter rye. Plants furnished with a complete nutrient solution and others with a solution containing one-tenth the amount of nitrogen in the complete solution produced 13.3 and 12.4 leaves, respectively, if germinated at 1° C., and 21 and 20.6 leaves, respectively, when germinated at 18° C. As a result of this experiment and of other experiments on the effects of potassium concentration and daylengths, PURVIS concluded that in winter rye "no known treatment can induce differentiation of flower initials before a certain minimal number of leaves has been formed." PURVIS and GREGORY (**14**) demonstrated later, however, that the required minimal number of leaves in winter rye could be altered within a certain range by vernalization or certain types of photoperiodic treatments. NUTMAN (**9**) found that rye grains weighing only 2.3 mg., obtained by early harvesting of the heads, produced plants with

[1] Contribution from the Department of Plant Sciences, University of Oklahoma, n.s. 93.

In this paper the expression "floral development" refers to that part of the reproductive cycle beginning with "floral initiation" (initiation of inflorescence primordia), and progressing through "exsertion" (appearance of the inflorescence above the surrounding leaf sheath) and "flowering" (anthesis).

approximately the same number of leaves as those grown from normal grains weighing about 36 mg. SHARMAN (**15**) removed the blades from the leaves of plants of a pure line of wheat successively as each one became expanded. These plants were markedly reduced in size in comparison with the controls, but there was practically no effect on the number of leaves produced before the initiation of inflorescences, the mean number being 12 for the controls and 11.4 for the defoliated plants. SHARMAN therefore suggested the use of the number of leaves on the main axis of such annual grasses as an index of the maturity of the individual at any stage.

Very little work has been done concerning the required minimal number of leaves on stems of perennial grasses before initiation of inflorescences. SHARMAN (**16**) reported that the number of leaves (counting the first leaf with a blade—the transition leaf—as the *first*) formed on a tiller of couch grass (*Agropyron repens* [L.] Beauv.) before initiation of the inflorescence on that tiller is ten or eleven. Moreover, he found that if a tiller has not formed the requisite number of leaves (and therefore attained sufficient maturity) by the time the daylength reaches a certain value, it apparently cannot initiate an inflorescence. OLMSTED (**11**) found that the number of elongated internodes (and presumably the number of leaves) on flowering culms in some strains of side-oats grama varied considerably with differences in photoperiod. In a Texas strain he found considerable variation in respect to the above characteristic even on natural daylength in the greenhouse at Chicago. Despite the somewhat opposite results reported above, it was thought desirable to attempt to correlate the number of expanded leaf blades with the stage of development of the inflorescence on the same stem in the five perennial grasses under investigation.

EVANS and ELY (**5**) found that the presence or absence of internodal elongation in grasses before the initiation of inflorescence primordia on the same stem is quite variable even in the same genus. In Kentucky bluegrass (*Poa pratensis* L.) and in timothy (*Phleum pratense* L.) there is essentially no internodal elongation of the stem until about the time its growing point enters the reproductive phase (**5, 6**). In Canada bluegrass (*Poa compressa* L.), on the other hand, "even the earliest internodes of the shoot become slightly elongated," so that the growing point is soon elevated somewhat above the ground level (**6**). The five grasses under investigation were therefore examined to determine if any internodal elongation occurred before initiation of the inflorescence on the same stem.

Little bluestem was studied in three topographic sites (southeast slope, ridgetop, and northwest slope) to determine whether there were obvious and consistent differences in regard to the above points in relation to slope exposure.

Material and methods

This project was carried out on the farm of C. H. TAYLOR (lat. 35°9′ N.) about 8 miles south of Norman, Oklahoma, during the growing season of 1948. The areas of study have never been in cultivation and have not been very heavily grazed for many years.

The general topography and vegetation are shown in figure 1. Little bluestem was the dominant grass in 1948. Big bluestem, Indian grass, switch grass, and side-oats grama were fairly sparsely scattered except for certain localized

areas, the first three species being most common on the north-facing slopes.

In order to obtain a relatively complete picture of the local and microclimatic conditions under which growth and floral development went on, the physical setup described below was used. Three plots, each about 25 × 25 feet—face, (*b*) soil temperature ($\frac{1}{2}$-inch depth) at 3:30 P.M. on one day of each week, (*c*) weekly precipitation (except on the plot on the northwest slope), and (*d*) total weekly evaporation from Livingston white and black atmometer spheres. The shade and support for one of the maximum-minimum thermometers are shown

FIGS. 1–2.—Fig. 1, general view showing topography and vegetation of locality in which study was made. Fig. 2, appearance of exclosure on southeast slope. (Photographs taken in September.)

one on a southeast slope of about 10° (fig. 2), one on a northwest slope, also of about 10°, and one on the level ridge-top between the slopes—were fenced to exclude cattle. Physical data taken on each plot (March 30–October 27) were: (*a*) the weekly maximum and minimum air temperatures about 1 foot above the soil surin place on one of the plots (fig. 3). Quart jars were used as reservoirs for the atmometers and were equipped with S-tubes with mercury valves as rain traps. The jars were placed in boxes in the ground, so that the spheres were about 6 inches above the soil surface.

Field observations on the growth of

big bluestem, switch grass, Indian grass, and side-oats grama were made each week; and, in addition, one specimen was collected from each of ten different plants of each species each week for dissection and microscopic examination to determine whether inflorescences had been initiated (6).

In the case of little bluestem only, separate observations were made on each topographic site to detect any differences in time of floral initiation, flowering, habit of growth, etc., within the species in these three habitats. One specimen was collected from each of five plants of this species from each site each week for dissection and microscopic examination. Admittedly, this sampling was not so comprehensive as would be desirable, but limitations of time prevented the dissection of more specimens each week.

FIG. 3.—Shade and support used for maximum-minimum thermometer.

Results

MICROCLIMATIC DATA

From the standpoint of water relations the 1948 growing season at the experimental location was unusually favorable through the week ending on August 11 (fig. 4). From August 11 to October 27, however, the total rainfall was only 0.67 inch. On the basis of a 53-year record at Norman, Oklahoma, the rainfall in March was 3.02 inches above normal; in April, 2.44 inches below normal; in May, 1.27 inches above normal; in June, 2.61 inches above normal; in July, 0.13 inch below normal; and in August, 1.83 inches below normal. In response to the abundant precipitation throughout the critical period in the summer, the highest yield of range grass seed in many years was produced.

The weekly maximum air temperature from May 5 through October 6 was usually slightly lower on the ridge-top than on either of the slopes (fig. 5). The breaks in the curves were due to damage to the thermometers during the weeks in which they occur. There was considerable variation as to the highest weekly maximum temperature on the southeast and northwest slopes, although the maximum was usually higher on the southeast slope throughout July, August, and September.

The weekly minimum air temperatures were usually practically identical on the various plots (fig. 5). Apparently a temperature inversion effect occurred during the week ending May 12.

The highest mean monthly soil temperatures on all plots (based on one determination a week) occurred in July, in contrast to the highest mean monthly maximum air temperatures, which were recorded in September (figs. 5, 6). The weekly soil temperature was usually lowest on the northwest slope, with that on the ridge-top being intermediate (fig. 6). Actually, there was very little difference in the observed values of soil temperatures on the three plots during the latter

half of May, all of June, and the first week of July.

April was the month with the highest rates of evaporation on all plots (fig. 7). The rates then began to decrease in general until the lowest weekly rates of the season were reached during the week ending July 14. After that an irregular increase began and continued until a secondary maximum was reached during the week ending September 8. Factors contributing to the high rates of evaporation during April were the high winds, the subnormal rainfall, and the low height of the vegetation; those primarily responsible for the secondary maximum in September were the low rainfall and high temperatures and the drying of mature vegetation, thus providing less cover and decreased transpiration around the spheres (figs. 4, 5). In general, the rates of evaporation were considerably greater on the southeast slope, followed by those on the ridge-top, and then by those on the northwest slope. In fact, beginning on July 14 the average rate of evaporation from the white sphere on the southeast slope was greater than that from the black sphere on each of the other plots. It is noteworthy that big bluestem, Indian grass, and switch grass are practically lacking on the southeast slope. It should also be pointed out that the direction of the prevailing wind during most of the growing season is from the southwest.

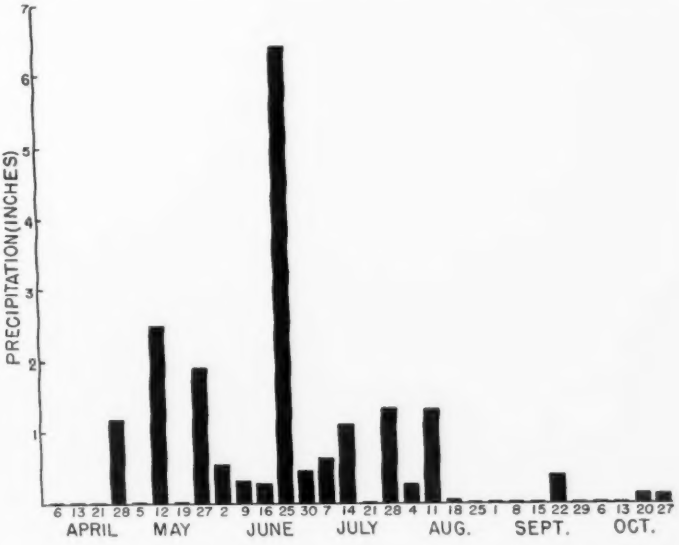

FIG. 4.—Weekly precipitation during thermal growing season on plot on southeast slope. Precipitation in rain gauge on plot on ridge-top was essentially same (plots about 100 yards apart). Numbers along horizontal axis represent weeks ending on those dates.

DATA ON GROWTH

In all species under investigation the aerial shoots which developed from axillary buds located at or below the soil surface on old culms or from rhizome buds usually had one or more scalelike leaves in addition to the prophyll. These were

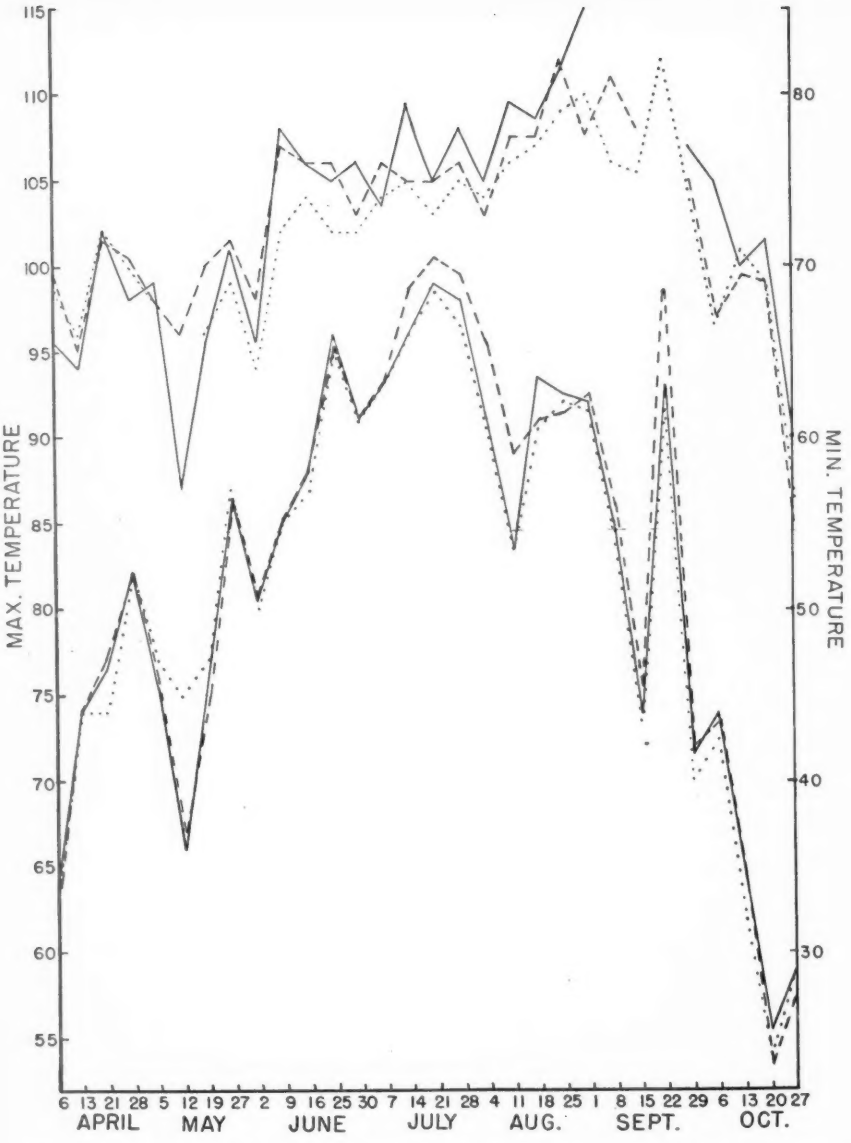

FIG. 5.—Weekly maximum (*three top lines*) and minimum (*three bottom lines*) air temperatures (F.) about 1 foot above surface of soil. *Solid lines*, southeast slope; *dotted lines*, ridge-top; *broken lines*, northwest slope. Abscissa as in fig. 4.

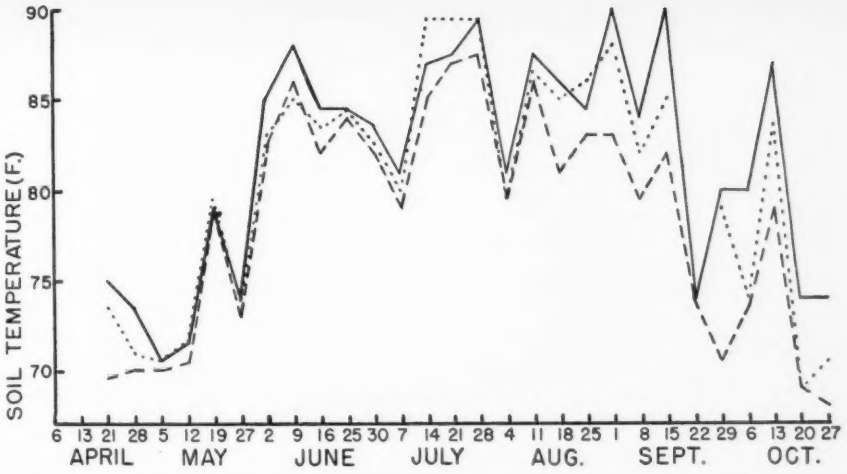

FIG. 6.—Soil temperatures on various topographic sites, taken at $\frac{1}{2}$-inch depth at 3:30 P.M. on one day of each week. Dates on horizontal axis represent days on which determinations were made. *Solid line*, southeast slope; *dotted line*, ridge-top; *broken line*, northwest slope.

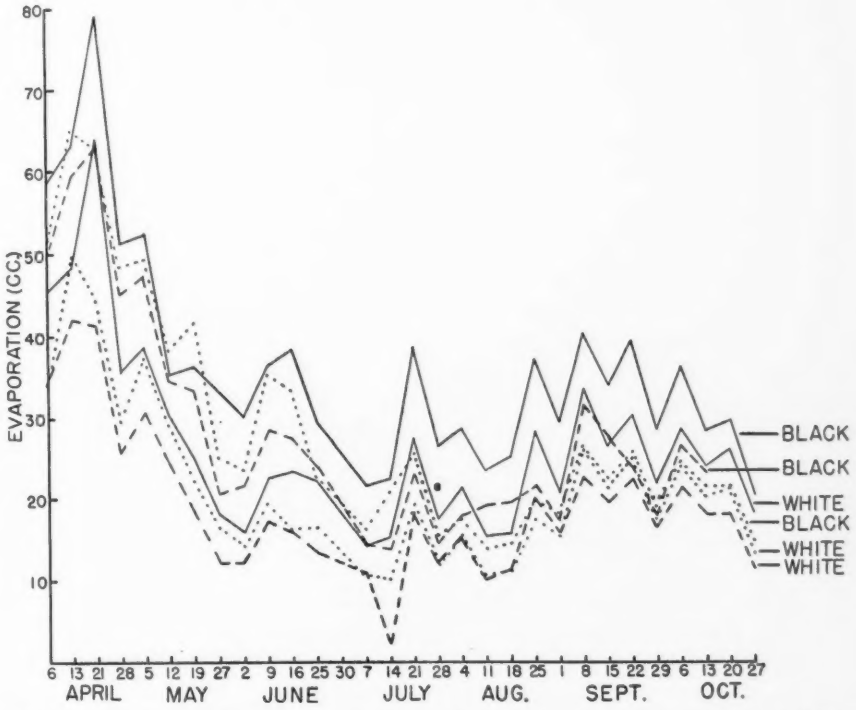

FIG. 7.—Average daily evaporation from white and black Livingston atmometer spheres. *Solid lines*, southeast slope; *dotted lines*, ridge-top; *broken lines*, northwest slope. Abscissa as in fig. 4.

followed by one or more leaves with reduced blades, the transitional leaves of SHARMAN (16). The first transitional leaf was counted as the *first* in all leaf counts that follow according to the practice of SHARMAN (16), and the internode below it was designated as the first internode.

LITTLE BLUESTEM.—This species showed the first visible signs of growth on March 30 on all sites. The new shoots apparently developed from axillary buds below the surface of the ground in most cases.

There was a slight elongation of the fourth, fifth, sixth, or seventh internodes or any combination of these in consecutive order even before the initiation of flower primordia. Among the tillers dissected and examined, the total amount of such elongation of these four internodes varied from almost none to as much as 40 mm., with the average total elongation being 14 mm. on the southeast slope and 15 mm. on each of the other sites.

The number of leaves with blades expanded—hereafter referred to as expanded leaves—at the beginning of floral initiation was either seven or eight on all stems counted. Conversely, before about July 14, practically all culms with as many as eight expanded leaves were found to have young inflorescences or to be in the microscopically visible transition stage. After July 14, however, culms bearing up to eleven expanded leaves were found with the growing points still in the vegetative state.

The total number of leaves initiated on a stem before floral initiation varied from fifteen to twenty on the southeast slope, fifteen to twenty-one on the ridge-top, and fourteen to twenty on the northwest slope. Of the stems observed on the southeast slope, 75% initiated either sixteen or seventeen leaves before the growing point was transformed into an inflorescence; on the ridge-top, 55% of the stems initiated sixteen or seventeen leaves; and on the northwest slope, 60% of the stems initiated sixteen or seventeen leaves. Thus, over half of the analyzed shoots of little bluestem on each topographic site formed either sixteen or seventeen leaves before initiation of the inflorescence.

The average maximum stem length (based on twenty mature flowering culms for each plot) was 103 cm. on the southeast slope, 106 cm. on the ridge-top, and 106 cm. on the northwest slope. There was virtually no significant elongation of any internodes below the fifth in mature culms, but, starting with the fifth, the average length of successive internodes increased up to and including the tenth, after which it decreased. This was true for plants from all plots (table 1). Average lengths of the fifth, sixth, seventh, and eighth internodes of plants on the ridge-top were approximately the same as of those on the northwest slope, but these lengths were considerably greater than those of the corresponding internodes of plants from the southeast slope (table 1). The same general trend was noted, also, for the fourteenth, fifteenth, and sixteenth internodes.

BIG BLUESTEM.—This species started growth a few days later in the spring than any other species studied, the first outward signs appearing about April 10.

Some internodal elongation had occurred when microscopically visible flower primordia were first detected, the mean total elongation, including all internodes, then being 12 mm. The amount varied from as little as 4 mm. to as high as 50 mm. in the interval of growth before the close of the period of floral initiation. Actually, a number of vegetative culms collected later in the season had a considerably greater amount of inter-

nodal elongation than the maximum mentioned above. Internodal elongation thus definitely can and does occur in big bluestem without the initiation of inflorescences.

The number of expanded leaves at floral initiation was nine to eleven. Until the end of the major period of floral initiation on about August 4, most of the culms with as many as eleven expanded leaves were found to have young inflorescences or to be in the transition stage. In the latter part of August, and even in October, however, some culms with as many as twelve expanded leaves were still in the vegetative state. This suggests that shoots which do not initiate inflorescences before a certain date remain vegetative.

The total number of leaves formed before floral initiation varied from fifteen to eighteen, with about 60% of the culms having sixteen or seventeen. Thus, the total number of leaves formed before initiation of the inflorescence in this species was essentially the same as in little bluestem, but the number of expanded leaves at that time was slightly higher.

The average length of each of the first four internodes, as in little bluestem, was less than 0.5 mm. Beginning with the fifth internode the average lengths of successive internodes increased up to and including the twelfth, after which they decreased (table 1).

TABLE 1

AVERAGE* LENGTHS (MM.) OF INTERNODES BASED ON TWENTY MATURE CULMS FOR EACH SPECIES. INTERNODE BELOW TRANSITION LEAF CONSIDERED AS FIRST (SEE TEXT)

NUMBER OF INTERNODE	LITTLE BLUESTEM			BIG BLUESTEM	SWITCH GRASS	INDIAN GRASS	SIDE-OATS GRAMA
	S.E. slope	Ridge-top	N.W. slope				
1	0	0	0	0	7	33	0
2	0	0	0	0	68	64	6
3	0	0	0	0	131	113	18
4	0	0	0	0	120	146	37
5	1	2	2	1	109	172	59
6	4	9	10	2	113	264	78
7	16	42	45	10	128	286	.....
8	79	83	84	52	.....	.....	.....
9	128	113	130	110	.....	.....	.....
10	144	128	146	155	.....	.....	.....
11	133	126	130	196	.....	.....	.....
12	116	112	113	212	.....	.....	.....
13	95	96	93	205	.....	.....	.....
14	79	81	80	173	.....	.....	.....
15	63	71	67	130	.....	.....	.....
16	48	61	55	105	.....	.....	.....

* A zero indicates any average elongation up to 0.5 mm.

SWITCH GRASS.—This species showed emergence of new shoots above ground about April 6. The first, second, and third internodes elongated slightly before any sign of an inflorescence became visible. Many culms which did not initiate inflorescences elongated considerably later in the season, as was the case with both little bluestem and big bluestem. Some such culms of switch grass showed a total internodal elongation of 32.5 cm.

The culms had either five or six (usually five) expanded leaves at the time of floral initiation. Until approximately the last of June any culm with as many as six expanded leaves usually had a well-developed inflorescence or was in the transition stage. This correlation, however, became less positive as the season progressed, suggesting that some shoots do not initiate inflorescences if they do not reach the correct stage of maturity by a certain time in the growing season. The total number of leaves formed before floral initiation in switch grass was either six or seven; 60% of the culms showed seven.

The average maximum length of twenty flowering culms was 124 cm. The situation regarding internodal elongation was unusual in this species. The length of successive internodes increased from the first to the third internode, decreased for the next two internodes, and then increased again to the top of the plant (table 1). A majority of individual culms showed this variation in internodal length from base to top.

INDIAN GRASS.—This species resumed top growth about April 6. The first green shoots were apparently derived from the terminal buds of shoots which started growth late in the previous growing season. The previous year's growth did not turn green except for the upper leaf. In such cases there were no newly formed scale leaves or transition leaves. The first leaf to show green was one on which the blade had partially expanded the previous growing season and which formed almost a completely new blade, apparently by resumption of growth at the base of the blade. For such shoots the leaf counts included those leaves formed during the previous year, starting with the first transitional leaf as usual. The shoots from rhizome buds did not appear aboveground until April 24.

Considerable internodal elongation occurred in this species before initiation of inflorescences. The actual total amount varied a great deal from culm to culm, but the average was about 65 mm. for those observed. There was no consistent difference between the two types of shoots described above in this respect.

The number of expanded leaves at the beginning of inflorescence initiation was three or four, regardless of the type of shoot, the smallest number of all species examined. This was true despite the fact that the total number of leaves formed prior to this time was as great as or greater than in switch grass and side-oats grama.

All internodes (counting that below the transition leaf as the first) elongated, and the lengths of successive internodes continued to increase to the top of the plant (table 1). The average maximum stem length, based on twenty mature flowering culms, was 156 cm.

SIDE-OATS GRAMA.—Some plants started growth about March 30. The majority of the shoots developed from axillary buds slightly below the surface of the ground, but some developed from rhizome buds.

Of the five species studied, side-oats grama was the only one which showed practically no internodal elongation prior to the initiation of inflorescences. In fact, unless floral initiation had occurred, virtually no culms elongated, even as late as October.

The number of expanded leaves at the time of floral initiation was five or six, while the total number of leaves formed before then was five to seven. Until the end of the major period of floral initiation practically every culm of side-oats grama

with as many as six expanded leaves was found to have a young inflorescence or to be in the transition stage. After this time, however, this indication of the stage of development was no longer reliable.

The average length of the first internode (internode below first transitional leaf) of mature culms was less than 0.5 mm. All other internodes elongated, however, and the average lengths of successive internodes were greater from the base to the top, as in Indian grass (table 1). The average maximum length of culms of side-oats grama was 62 cm., this being the shortest of the species investigated.

Data on floral development

Data concerning floral development in all species investigated are given in table 2. The period of major floral initiation was that interval during which practically every culm with the requisite minimum number of leaves had a young inflorescence or was in the transition stage. The daylength given was the time from sunrise to sunset plus 1 hour for dawn and twilight, following the practice of Olmsted (10).

There were no apparent differences in the flowering data for little bluestem on the three topographic sites; therefore, they were averaged.

TABLE 2

Flowering data. All dates represent weeks ending on given dates

	Little bluestem	Big bluestem	Switch grass	Indian grass	Side-oats grama
Initiation of first flower primordia	June 16	June 30	May 27	June 16	May 19
Period of major floral initiation	June 25–July 14	July 14–Aug. 4	May 27–June 16	June 25–July 14	May 27–July 7
Beginning of first exsertion	July 28	Aug. 18	June 30	Aug. 18	June 2
Days from start of floral initiation to start of first exsertion	43	50	35	64	15
First flowering (anthesis)	Aug. 4	Aug. 25	July 21	Aug. 25	June 9
Time from beginning of exsertion to anthesis	Less than 1 week	Less than 1 week	About 4 weeks	Less than 1 week	About 2 weeks
Major flowering period	Aug. 25–Sept. 15	Sept. 1–15	Aug. 11–Sept. 1	Sept. 1–15	June 16–July 28

Time in life-history	Daylengths*									
	Hr.	Min.	Hr.	Min.	Hr.	Min.	Hr.	Min.	Hr.	Min.
Initiation of first flower primordia	15	33	15	33	15	19	15	33	15	8
Start of major period of floral initiation	15	33	15	21	15	19	15	33	15	19
End of major period of floral initiation	15	21	14	51	15	33	15	21	15	28
Beginning of first exsertion	15	3	14	25	15	32	14	25	15	25
First flowering	14	51	14	10	15	13	14	10	15	30
Start of major flowering period	14	10	13	55	14	38	13	55	15	33
End of major flowering period	13	24	13	24	13	55	13	24	15	3

* Sunrise to sunset plus 1 hour for dawn and twilight.

The number of days from the beginning of floral initiation to the start of first exsertion varied from as few as 15 days in side-oats grama to as many as 64 days in Indian grass; and the time from the beginning of exsertion to anthesis varied from less than 1 week in three of the grasses to 4 weeks in switch grass. These data demonstrate how imperative it is that the criterion used to determine flowering (initiation, exsertion, or anthesis) be stated clearly in any paper concerning the dates of flowering of grasses.

In all species under investigation except side-oats grama, the difference between the daylength midway in the major period of floral initiation and that midway in the major flowering period was 1–2 hours. These differences may indicate differences in photoperiodic requirements for floral initiation and anthesis in the individuals of little bluestem, big bluestem, Indian grass, and switch grass observed. In the case of side-oats grama there was essentially no difference between the daylength midway in the major period of floral initiation and that midway in the major flowering period. This suggests that the optimum photoperiod for floral initiation may be the same as that for flowering in most plants of this species observed in the present investigation. Neither the major period of inflorescence initiation nor the major flowering period was so well defined in side-oats grama as in the other species; throughout the summer a few scattered culms were found to be initiating inflorescences in each week's collection.

Discussion

In the growing season of 1948 on the plots at Norman, Oklahoma, all species under investigation started growth within a period of slightly over 1 week. The fact that all species started growth at essentially the same time may have been the result of the weather conditions during March. A severe blizzard occurred early in the month, and the temperature remained subnormal until about the middle of the month, after which it increased rapidly. The last week was quite warm, resulting in soil temperatures which were satisfactory for the growth of all five species.

Side-oats grama was the only species investigated which showed practically no internodal elongation before the microscopically visible transformation of the vegetative growing point to the floral developmental phase. This lack of internodal elongation before initiation of an inflorescence held true throughout the growing season in side-oats grama. On the other hand, OLMSTED (**10**) reported that some of the plants of strains of side-oats grama from El Reno, Oklahoma, and Beaver County, Oklahoma, elongated on a 9-hour day, although they did not flower on such a photoperiod and apparently did not form floral primordia. Moreover, he found that internodal elongation of the primary axis did not indicate reproductive activity at all in strains from southern Texas and southern Arizona.

Considerable attention has been paid to the pattern of internodal elongation in various grasses. PRAT (**12**) stated: "It is a conspicuous fact that the internodes of cereal straw increase regularly in size from the base to the top. This peculiarity seems to be an expression of the special polarity of the stem, caused by the presence of the ear at its apex." He stated further that "wild species of grasses show characters similar to those of cultivated cereals." In commenting on the results of an investigation of *Poa nemoralis* L., PRAT wrote: "The aerial and reproductive parts of its stem are thus built ac-

cording to the same general rule as a cereal straw. But it is worthy of notice that, in the perennial species, the basal part which is characterized by the presence of lateral shoots and short internodes, shows no definite law of length. In this inferior vegetative portion, the elongation is governed only by the antagonism of growth between internodes and shoots, by the importance of rooting of the nodes, etc." In the investigated plants of little bluestem, big bluestem, and switch grass there was no regular increase in the length of the internodes from the base to the top of a culm (table 1). All internodes from the tenth to the top in little bluestem and all internodes from the twelfth to the top in big bluestem were successively shorter on the average. In this connection it should be pointed out that the majority of the flowering culms of little bluestem had from one to three branches with inflorescences at every node from the tenth to the top. In big bluestem the majority of the flowering culms had lateral branches with inflorescences at all nodes from the twelfth to the top. No such morphological feature was correlated with the variation in switch grass.

The attainment of a definite minimum number of expanded leaf blades per culm was a fairly accurate criterion of the time of initiation of the inflorescence in all five species. The fact that a culm showed such a minimum number of expanded blades, however, did not necessarily mean that its growing point had changed to the reproductive phase in all cases; but no culm with a smaller number of expanded leaves (than the minimum specified) was found to have a young inflorescence or to be in the transition stage. In all except side-oats grama there was considerable evidence that any culm which does not have the minimum number of expanded leaves by the time the photoperiod reaches a certain length does not initiate an inflorescence. It is not suggested that the minimum numbers given for the various species would be true for all their strains or for the same strains under all conditions of growth. It is suggested that such a criterion would be practical and helpful in range management and in the production of native grass seed if it were demonstrated to hold for the strains of a species in a given locality over a period of years.

Little bluestem from the different topographic sites did not show any definite and constant variations. Flowering data on all plots were practically identical. Plants on the southeast slope were slightly shorter than the others; this may have been associated with the significantly greater rate of evaporation on that plot. The pattern of elongation in little bluestem was exactly the same on all plots. The average lengths of the fifth, sixth, seventh, and eighth internodes, however, and of the fourteenth, fifteenth, and sixteenth internodes were less on the plants of that species growing on the southeast slope than on the other sites. Again, this variation may have been associated with less favorable water relations on the southeast slope, but the fact that only the basal and top internodes were shorter suggests a genetic difference.

Larsen (7) investigated the photoperiodic responses of twelve geographic strains of little bluestem. Two of the strains originated from Oklahoma: one from Ardmore (lat. 34°10′ N.) and one from Arnett (lat. 36°10′ N.). In neither of these strains did the clonal divisions flower when grown continuously on a 13-hour photoperiod; both flowered on a 14-hour photoperiod; and the strain from Arnett flowered on a 15-hour photoperiod. The latter strain, however, was found

to be delayed in flowering on the 15-hour photoperiod, the time between the first evidence of elongation and first flowering being 56 days on a 15-hour photoperiod in contrast to 37 days on a 14-hour photoperiod. LARSEN suggested that his plants from Oklahoma might be intermediate-day plants with a lower critical photoperiod of between 13 and 14 hours and an upper critical photoperiod of around 15 hours. The data obtained for the plants observed by the writer under natural growing conditions (lat. 35°9′ N.) would not negate LARSEN's conclusions, because flowering did not occur until the daylength had decreased to 14 hours, 51 minutes, and their major flowering period did not begin until the effective daylength had decreased to 14 hours, 10 minutes (table 2). Moreover, the major flowering period ended by the time the daylength had decreased to 13 hours, 24 minutes. LARSEN found that two other strains of southern origin—one from Alexandria, Louisiana, and one from Durham, North Carolina—initiated floral primordia on a 15-hour photoperiod but did not flower. This might suggest that the photoperiodic requirements for flowering in those strains were different from those for initiation of floral primordia, because those same strains flowered well on a 14-hour photoperiod. A similar situation is indicated for the plants observed in the present investigation, because the daylength midway in the major period of floral initiation was 15 hours, 29 minutes, while the effective daylength midway in the major flowering period was 13 hours, 47 minutes. Only experiments under controlled conditions could prove definitely whether the above suggestion is correct. Such a project covering most of the present species is contemplated. Data concerning photoperiodic status as obtained by the methods used in the present investigation can serve only as a check, under natural conditions, of those conclusions which have been based on controlled photoperiodic experiments. In regard to the possibility suggested above, it is known that certain chrysanthemums require different photoperiods for flowering than they do for the initiation of flower primordia (**2**). Moreover, PURVIS (**13**) found that, in the case of winter rye after germination at 18° C., exposure to short days leads to earlier differentiation of flower primordia than does exposure to long days. In such cases, however, after differentiation of flower primordia has begun, further development is always hastened by long days. In some other species, flower primordia may be initiated under a wide range of photoperiods, but flowering will occur only over a more limited range (**4, 8, 17, 18**).

Data concerning floral initiation and flowering in observed plants of big bluestem, switch grass, and Indian grass were remarkably similar to those for little bluestem; therefore, the suggestion concerning differences in photoperiodic requirements for inflorescence initiation and anthesis would apparently apply similarly. Present data are not sufficient to place the plants in any definite photoperiodic category. They do *suggest*, however, an intermediate- or short-day classification in regard to flowering for all observed plants of the three former species. No study has been reported concerning responses of geographical strains of these three species to photoperiod. BENEDICT (**3**) classified big bluestem as a short-day plant on the basis of a limited number of tests on plants grown from seeds of unspecified origin. He investigated the photoperiodic responses of switch grass of unspecified origin also and concluded that "*Panicum virgatum* is a short-day plant." These conclusions were

perhaps unfortunate, because OLMSTED (10) and LARSEN (7) showed definitely that photoperiodic responses among different geographical strains of widely ranging native species of grasses varied considerably. ALLARD and EVANS (1) reported that the optimum range of photoperiods for flowering in plants of Indian grass originating in Virginia was 13.5–14.5 hours. This optimum range is slightly higher than the range in which the Oklahoma plants flowered. On the basis of the results of OLMSTED (10) and LARSEN (7) on other species, such a difference is not surprising because the latitude of the point of origin is less in the Oklahoma plants.

Present data suggest that the majority of plants of side-oats grama under investigation were either long-day or intermediate-day plants. Their major flowering period began about June 16, when the daylength was almost at its maximum (15 hours, 33 minutes), and ended when the daylength had decreased to 15 hours, 3 minutes. OLMSTED (10) investigated the responses of two strains from Oklahoma: one from El Reno (lat. $35\frac{1}{2}°$ N.) and one from Beaver County (lat. 37° N.). No plants flowered on a 9-hour photoperiod, while numerous individuals of these strains flowered on 16- and 20-hour photoperiods. Flowering among plants of these strains did occur on the 13-hour photoperiod but was more limited than on the longer light periods. OLMSTED suggested that these strains might include both intermediate- and long-day plants. The period between floral initiation and anthesis was much shorter in the present plants of side-oats grama than in any of the other four species investigated, so that both floral initiation and flowering occurred chiefly during the longest days of the growing season.

The length of time from the start of inflorescence initiation to exsertion was somewhat different for each species. In three species—little bluestem, big bluestem, and Indian grass—anthesis was found to begin in less than a week after the start of exsertion; this period was about 4 weeks long in switch grass and 2 weeks long in side-oats grama. These data can lead to some interesting speculations. It may be that the lengths of these periods are controlled by different photoperiodic requirements for floral initiation, culm elongation, and anthesis, at least in some species. Such a theory certainly would apply quite well to all except side-oats grama. In little bluestem, big bluestem, and Indian grass the optimum daylength for internodal elongation might be close to that for anthesis. Certainly, the lengths of the periods from inflorescence initiation to exsertion were considerably longer in the plants of these species than in those of switch grass. In plants of the latter, the daylengths at the time of exsertion were essentially the same as those during floral initiation, while those at anthesis were considerably different. This might possibly have been due to similar photoperiodic requirements for floral initiation and internodal elongation in the plants of this species and to different photoperiodic requirements for flowering. A study of the effects of various photoperiods on the lengths of the intervals mentioned above should be interesting.

Summary

1. Growth and floral development of little bluestem (*Andropogon scoparius*), big bluestem (*A. furcatus*), switch grass (*Panicum virgatum*), Indian grass (*Sorghastrum nutans*), and side-oats grama (*Bouteloua curtipendula*) under prairie conditions were investigated on the farm

of C. H. TAYLOR (lat. 35°9′ N.) near Norman, Oklahoma, during the growing season of 1948. Three plots—one on a ridge-top, one on a southeast slope, and one on a northwest slope—were fenced to exclude cattle. In addition to field observations on the condition of the five species, one culm was collected from each of ten different plants of each species (except little bluestem) each week for dissection and microscopic examination to determine whether inflorescences were being formed. For little bluestem, one culm was collected from each of five plants on each site each week to determine whether there were differences in growth and floral development on the various sites. Fairly complete microclimatic data were obtained for each plot.

2. All species started growth within a period of slightly over a week. Little bluestem and side-oats grama were earliest, showing the first signs of growth about March 30.

3. The number of leaves with blades expanded—hereafter referred to as expanded leaves—on a given culm was found to be a fairly accurate index of the time of floral initiation in all species up to the end of the major period of inflorescence initiation for each species. This number—counting the first leaf with a blade as the first—was seven or eight in little bluestem, nine to eleven in big bluestem, five or six in switch grass, three or four in Indian grass, and five or six in side-oats grama. In all except side-oats grama, there was considerable evidence that any culm which does not have the number of expanded leaves specified for the species by a certain date does not initiate an inflorescence. Under such conditions the growing point apparently remains vegetative and continues to initiate leaves. Such a criterion, if thoroughly worked out for the various species in a given locality, could be of considerable practical value to anyone who wishes to use a particular plot of grassland for both grazing and seed production.

4. Side-oats grama was the only species which showed practically no internodal elongation before floral initiation. Indian grass showed the greatest amount of such elongation.

5. Little bluestem did not show any very definite variations among the different topographic sites. The plants were shorter on the average on the southeast slope, but the pattern of elongation was exactly the same on all plots. Flowering data for little bluestem were essentially the same on all plots also.

6. Floral initiation proceeded at the greatest rate during the long days of late spring and early summer in all species. Flowering occurred chiefly during the long days of early summer in side-oats grama, but in the other species it did not begin until the daylength had decreased a substantial amount below that at the time of floral initiation. Considering the fact that all these grasses have previously been shown to be photoperiodically sensitive, using flowering as a criterion, the present data suggest a long- or intermediate-day flowering status for the plants of side-oats grama at Norman and an intermediate- or short-day flowering status for those of the other species investigated. They also suggest either that, with the exception of side-oats grama, the optimum photoperiod for floral initiation is different from that for flowering in the particular plants observed or that inflorescence initiation can occur over a greater range of photoperiods than it does under these field conditions.

7. The time between inflorescence initiation and exsertion varied with each species, being approximately as follows

in each case: 43 days in little bluestem, 50 days in big bluestem, 35 days in switch grass, 64 days in Indian grass, and only 15 days in side-oats grama. The time between the start of exsertion in a given culm and its flowering (anthesis) was less than 1 week in little bluestem, big bluestem, and Indian grass; but it was about 4 weeks in switch grass and 2 weeks in side-oats grama. The total time between inflorescence initiation and flowering, therefore, was just slightly less than 2 months in little bluestem and big bluestem, slightly over 2 months in switch grass and Indian grass, and about 1 month in side-oats grama. An investigation as to the effects of various constant daylengths on the above periods should be of value in explaining the observed variations.

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INTERNAL SUBERIZATION OF TISSUES

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Introduction

The continuation of the surface cuticle into the respiratory chambers of stomata and also into the immediately adjacent intercellular spaces of the mesophyll was noted by VON MOHL (**23**) more than 100 years ago and was confirmed by extensive observations of later anatomists (**9**). Seventeen years ago ARZT (**2**) demonstrated, in the leaves of dicotyledons, the occurrence of a visible "inner cuticle" about 1 μ in thickness and grading downward to the limit of microscopic visibility. More recently HÄUSERMANN (**8**) analyzed the results of quantitative experiments on the rise of lipophilic and hydrophilic liquids in narrow strips of *Dianthus* leaves and concluded that the internal surface of the leaf is coated with a layer of fatty material, probably cutin. Despite these observations, the occurrence of a lining of cutin, suberin, or fatty material on the internal surface of leaves does not appear to have been accepted as a current working concept in plant physiology (**10**).

In the present paper some results of investigations on such internal suberization, outlined in a previous note (**19**), are presented in greater detail. Suberin is more widely distributed in the plant than cutin, since it has been identified in deeper tissues such as the endodermis (**14**). Pending detailed chemical analysis, the terms "suberin" and "internal suberization" are temporarily retained. Suberin, as used in the present paper, connotes a wall material which is distinguished primarily by its resistance to the action of strong sulfuric and chromic acids.

Material and methods

A survey was made of various types of leaves: xerophytic, mesophytic, and hydrophytic, a list of which follows: HYDROPHYTES—*Eichhornia crassipes*, *Iris pseudacorus*, *Jussiaea repens*, *Nymphaea alba*, and *Potamogeton* sp. MESOPHYTES—*Acanthus mollis*, *Acer macrophyllum*, *Aesculus californica*, *Annona cherimola*, *Araucaria imbricata*, *Bergenia cordifolia*, *Brachychiton discolor*, *Casimiroa edulis*, *Ceratonia siliqua*, *Citrus sinensis*, *Clivia miniata*, *Cucurbita pepo*, *Cymbidium* hydrid, *Cynara scolymus*, *Echinocystis macrocarpa*, *Epidendrum* sp., *Feijoia sellowiana*, *Impatiens balsamina*, *Juglans californica*, *Mangifera indica*, *Musa nana*, *Macadamia ternifolia*, *Nerium oleander*, *Persea americana*, *Pelargonium* sp., *Pinus coulteri*, *P. ponderosa*, *Platanus racemosa*, *Psidium guajava*, *Pteridium aquilinum*, *Ricinus communis*, *Venegazia carpesiodes*, and *Vicia faba*. XEROPHYTES—*Aeonium arboreum*, *Asclepias subulata*, *Cercidium floridum*, *Crassula arborescens*, *Dalea spinosa*, *Encelia farinosa*, *Selaginella bigelovii*, and *Thamnosoma montana*.

The stems selected for intensive examination included those of the angiosperms *Acanthus mollis*, *Juglans californica*, *Platanus racemosa*, and *Ricinus communis* and of the gymnosperms *Pinus coulteri* and *P. ponderosa*.

Seedlings and mature plants of *Ricinus communis* and of *Vicia faba* provided excellent material for the study of roots.

The flowers of *Pelargonium* were compared with those of the orchids *Epidendrum* and *Cymbidium*, while the pod of *Vicia faba* was used in the examination of a typical fruit.

Suberin lamellae (**20**) appear as translucent varnish-like layers in cell walls which are not shrunken by alcohol or other fixatives. Sections of fresh material—transverse, radial, tangential, and paradermal as required—were examined in water, in glycerin mounts, and also during and after irrigation with various microchemical reagents.

The most satisfactory method so far found for the identification of suberin lamellae is the standard cellulose test IKI-H_2SO_4. Membranes lining intercellular spaces and intracellular pellicles stain clear yellow with IKI. On irrigation with H_2SO_4 (80%) the cellulose gradually swells, turns blue, and finally disintegrates, leaving clear a more or less complete network of shrunken protoplasts and remains of interconnecting plasmodesmata. In the meantime the suberin lamellae turn dark brown, wrinkle, may buckle away from the swelling wall, and, if thin, ultimately fragment. If, however, the lamellae are thick, then fragments of network persist. When suberin is exceptionally heavy and impregnates the entire cell wall, the usual swelling of the wall and the accompanying blue coloration are almost completely suppressed. Thus, at the end of the reaction, after prolonged irrigation with strong H_2SO_4, the cell walls emerge darkened in color but intact in outline.

Lignin, like suberin, darkens in H_2SO_4 but, unlike cellulose, does not disintegrate. Lignin and suberin may be distinguished by their relative solubilities in strong chromic acid (50%). The latter reagent sooner or later breaks down all wall constituents except suberin and cutin (**5**). As in the usual maceration techniques, pectic substances disappear first, followed by cellulose and lignin, simultaneously with the protoplasts. The process of disintegration may be observed and arrested at any point by irrigation first with water and later with Sudan III. Cutin and suberin are stained, the intensity of color being dependent on the thickness of the layer. The emission of bubbles during the chromic acid reaction is always more or less troublesome.

A method of biological assay, by the action of cellulose bacteria, provides further evidence of the presence of suberin lamellae. Leaf segments or paradermal sections resting on agar in Petri dishes are suffused with a mixed culture of cellulose bacteria.[1] Within about a week or less the mesophyll is isolated from both upper and lower epidermis. As the cellulose of the palisade and spongy tissue is broken down by bacterial action, the cell outlines may still be partially traced by the indestructible fragments of lamellae stainable in Sudan III.

Numerous other reagents and staining methods were tried out with variable success. Prolonged soaking (7–10 days) in chlor-zinc-iodine (**2**) proved effective with certain woody sections. The membrane in general is too thin to stain deeply with Sudan III or Sudan IV. Osmic acid does not differentiate clearly between suberin lamella and cellulose. Eosin, thionin, gentian violet, anilin blue, and other common stains were occasionally found to be useful, but IKI, followed or not by H_2SO_4, remains the most immediately effective reagent.

Preliminary observations were made with Shillaber's liquids of varying refractive index. The suberin lamella was

[1] I am indebted to Dr. JANN of the Bacteriology Department for the preparation of these cultures.

clearly defined in liquids of refractive indexes 1.4 and 1.5. This method appears to have possibilities for further detailed study.

Observations

Leaf

The growth of a leaf, a continuous process, may be arbitrarily considered in four stages: (*a*) resting bud and meristematic primordia; (*b*) expanding leaf; (*c*) maturing leaf; and (*d*) leaf-fall (**20**). The first three stages are paralleled by corresponding structural changes in the stem subtending the leaf. In the final stage some growth of tissue may occur at the junction of leaf base and stem prior to abscission and during scar formation. As any leaf ages, it changes from a lighter to a darker green and also from a softer to a harder texture. Hardness results in part from increased thickness of cuticle and in part from increased internal suberization.

Internal suberization of leaves varies with the age, the habit, and the habitat of the plant. Thus, in all young leaves and in the mature leaves of hydrophytes and of some shade mesophytes, the suberization may be termed weak. The first traces of suberin deposition appear during the early stage of leaf expansion when palisade and spongy parenchyma are not yet clearly defined. At this time thin pellicles in the still minute intercellular spaces may be clearly stained with IKI. During subsequent growth the pellicles keep pace with the enlarging intercellular spaces and, when the leaf is fully expanded, may measure about 1 μ in thickness. Meanwhile, a film appears which lines the inner surface of the cell wall and resembles a tertiary lamella. When first detected it is very thin but is distinct from the plasma membrane and, optically and microchemically, so far as determined, is indistinguishable from the material of the envelope of the intercellular spaces (figs. 1, 7, 8).

The degree of suberization is clearly indicated by treatment with IKI-H_2SO_4. In weakly suberized leaves all suberin films are thin but stain clearly with the IKI. On irrigation with H_2SO_4 the cellulose of the walls swells, turns blue, and ultimately disintegrates. Meanwhile the suberin lamellae darken, wrinkle, appear beaded, and ultimately break down into insoluble fragments which persist as faint tide marks outlining the network of shrunken protoplasts, plasmodesmata, and intercellular spaces (figs. 2–5).

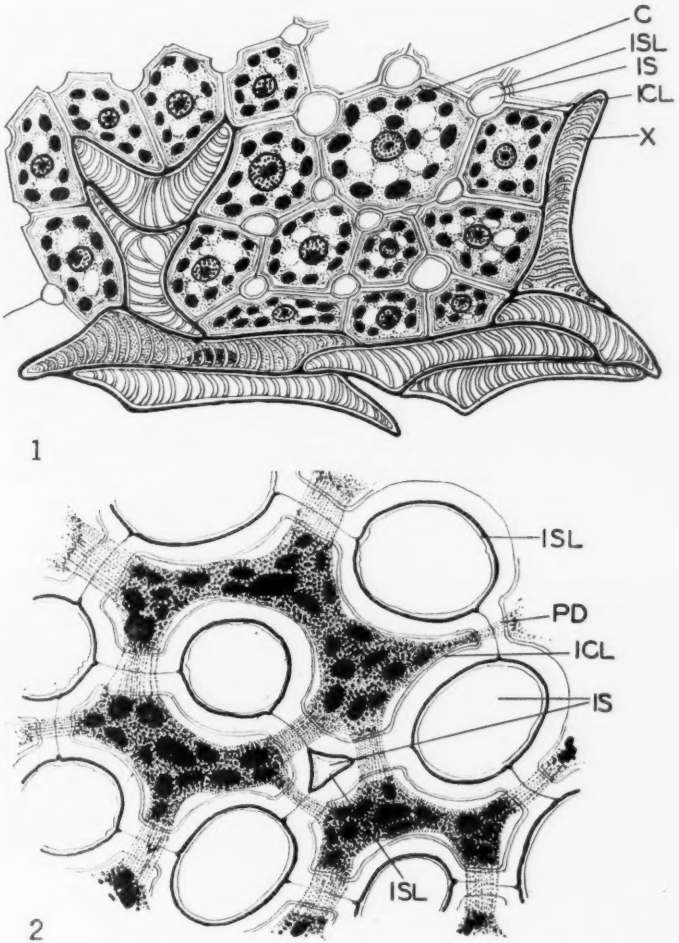
What may be termed intermediate suberization occurs in older leaves in many deciduous and some evergreen mesophytes and also in the transitory reduced leaves of certain xerophytes. Suberin in the young leaves is as described but later appears to extend outward as a film from the intercellular spaces for variable distances along both surfaces of the middle lamella. At the same time both intercellular and intracellular lamellae become more conspicuous. On treatment with IKI-H_2SO_4, the usual swelling, blue coloration, and eventual disintegration of the cellulose result, while the intracellular suberin pellicles wrinkle and may become somewhat distorted but remain unbroken. The ensheathed middle lamella may disintegrate as during maceration, leaving thin suberin lamellae on both sides. If, however, suberization is more intense, approximating that described in the following paragraph, middle lamella and suberin sheaths are completely fused and consequently persist unified and intact (figs. 9–12).

Where suberization is strong, as in certain mesophytes, and in all xerophytes examined except the succulents, the entire wall is impregnated with suberin.

On addition of IKI-H_2SO_4 wall swelling is negligible, and the blue colorization of cellulose is almost completely masked by the dark brown of the suberin. Thus, at the end of the reaction the cell outlines of both spongy and palisade parenchyma remain practically unchanged (fig. 6). The micropapillate condition of the surface seen in *Platanus* appears to be general. The papillae are similar in size to the beads seen in weaker suberin films prior to disintegration.

Stem

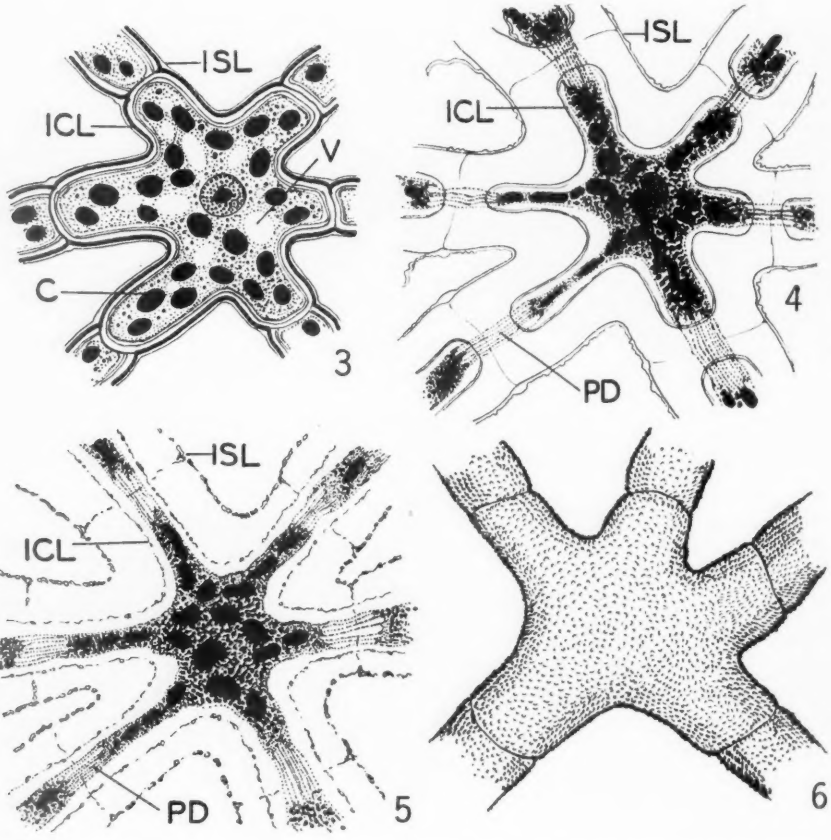
Angiosperm.—Since suberin is deposited during the early stages of leaf expansion, it was expected and confirmed that similar deposition occurs in the tissues of



Figs. 1–2.—Paradermal sections through spongy parenchyma, during IKI-H_2SO_4 irrigation, showing internal suberization. *C*, chloroplast; *ICL*, intracellular lamella; *ISL*, intercellular space lamella; *IS*, intercellular space; *PD*, plasmodesmata; *X*, spiral vessels. Fig. 1, *Platanus racemosa*, young leaf. Fig. 2, *Persea americana*, full grown but still soft leaf.

both vegetative and inflorescence axes. In the elongating internodes of *Ricinus communis*, *Platanus racemosa*, *Juglans californica*, and other species, suberin appears in the intercellular spaces of the differentiating cortex, pith, and vascular tissues, simultaneously with and at the nodal level of the developing leaves. The pellicles of suberin are extremely thin, but, as in the mesophytic leaf, they may be clearly stained with IKI. Sooner or later intracellular lamellae distinct from the plasma membranes also appear (fig. 13).

In the vascular system suberization occurs in both primary and secondary phloem and xylem. The differentiation of xylem and phloem elements in the leaf is similar to that in the stem. In the latter, intercellular spaces are small but are much more readily seen in fresh material than in preserved, and therefore more or



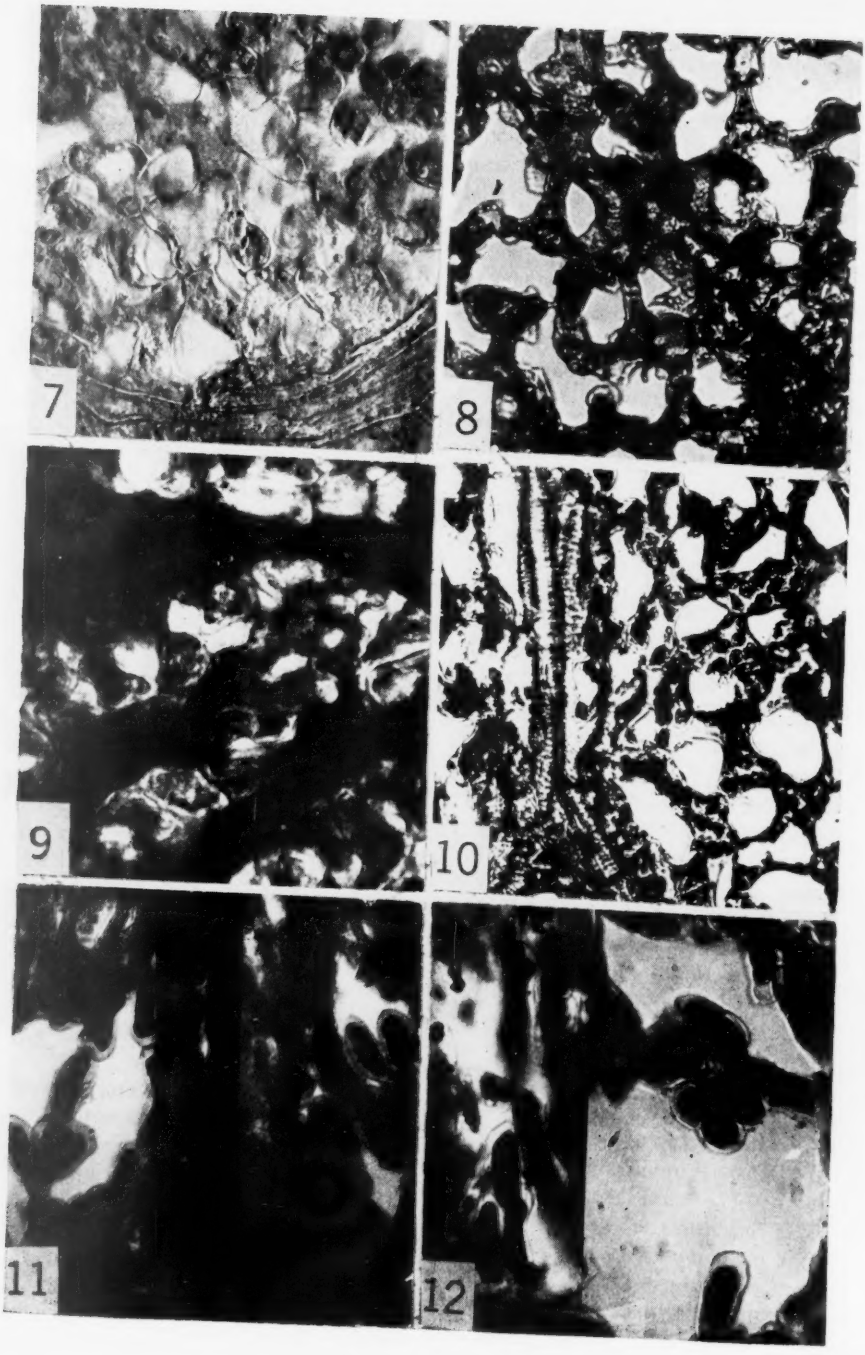
FIGS. 3–6.—Paradermal sections showing typical mesophyll cells during and after IKI-H_2SO_4 irrigation. *C*, chloroplast; *ICL*, intracellular lamella; *ISL*, intercellular space lamella; *IS*, intercellular space; *V*, vacuole. Figs. 3–5, *Acanthus mollis*, mature leaf. Suberin lamella, change from smooth to wrinkled and fragmented condition. Fig. 6, *Platanus racemosa*, mature leaf. Characteristic micropapillate surface of cell after irrigation.

less deaerated, tissue. In the phloem, both primary and secondary, of *Ricinus* and other species, intercellular and intracellular lamellae are clearly evident on staining with IKI, appearing between and within sieve tubes, companion cells, phloem fibers, parenchyma, and rays (figs. 14, 15).

In the xylem of *Ricinus* the stages of suberization may be followed during differentiation. The comparatively wide spiral elements are connected with adjacent cells, whether other spiral elements or xylem parenchyma, by plasmodesmata. By the time the differentiating spiral is lignified, a very thin, refringent, clearly stainable pellicle lines the entire inner surface of the cell (fig. 16). At this time also suberin is evident in the minute intercellular spaces and appears to extend outward from them as a film along both surfaces of the middle lamella. When the protoplast dies, the plasmodesmata apparently retreat from the vessel wall and, as they do so, seal behind them the vacant pores. Thus the entire vessel is coated, within and without, by a continuous layer of fatty substance. As only the spiral thickening of such a vessel is lignified, the cellulose disappears on treatment with IKI-H_2SO_4, leaving behind a two-layered envelope.

Ricinus, *Platanus*, and *Juglans* provide excellent material for the study of suberization in secondary xylem. The pattern of suberization in vessels and other elements is in general similar throughout the dicotyledons. Certain phases of the structure of *Ricinus* have been discussed previously (**16, 17**). The anatomy of *Platanus racemosa* and of *Juglans californica* is essentially similar to that of *Platanus occidentalis* and of *Juglans nigra* (**4**). The differentiating vessel segment begins to expand as soon as it is cut off from the parent cambial cell (figs. 17–27). In surface view the cell wall at this stage appears as a cellulose net, the roughly hexagonal meshes of which are perforated by closely set plasmodesmata less than 1 μ in diameter. In profile, the plasmodesmata are observed connecting the protoplasts of the adjacent differentiating cells: vessel segments, fibers, xylem parenchyma, or rays. Deposition of the secondary wall becomes marked when the vessel segment reaches its maximum diameter. About this time extracellular and intracellular suberin layers become visible. Intercellular spaces are small but detectable in the differentiating xylem, and suberization appears to begin, as in the leaf mesophyll, in these areas of strain at the cell corners, and to extend from here along the middle lamellae, forming the extracellular suberin layer. Meantime the intracellular pellicle now becomes visible and resembles in appearance, though not in origin, a tertiary lamella. As the walls continue to thicken, the openings of the pits are narrowed by the overhang of the cell wall, and sooner or later lignification occurs. The intracellular suberin pellicle then increases considerably in thickness. As the end walls of the vessel segment disappear and the protoplast dies, the plasmodesmata withdraw from the pit areas but leave behind a membrane which seals the base of the pit. This withdrawal presumably explains the absence of plasmodesmata in walls of lignified elements. The mature xylem vessel is thus completely surfaced and lined throughout its length by suberized envelopes.

In many sections mounted in water or glycerin, or stained with IKI only, the inner surface of the xylem elements appears to be micropitted. This means that the surface of the protoplast is micropapillate and therefore has an enormous-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ly increased surface area. It may be that from these micropapillae of the plasma membrane micro-plasmodesmata extend into the developing vessel walls. These strands, if present, may be detectable by means of the electron microscope. The same micropitting is noted occasionally in parenchymatous cells so that the micropapillate condition of the plasma membrane may be general.

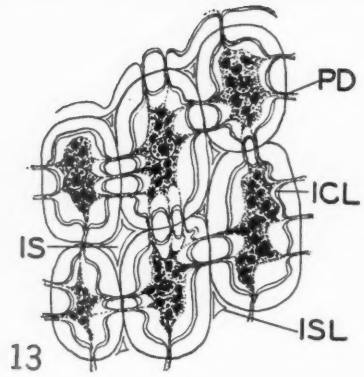
The effect of internal suberization on sap transport was studied in the stem of *Juglans* (figs. 34–35). After bud break in spring and while the bark was slipping, young, vigorous, leafy, basal suckers were bent down into a solution of eosin and India ink and cut under water. The stems were sectioned within half an hour after cutting and again at later intervals. When they were first examined, India ink was evident in the lumen of the empty vessels, and the walls, particularly the translucent suberin lamellae, were more or less clearly stained by eosin. In marked contrast to this the adjacent cells—fibers, rays, or parenchyma—showed no trace of eosin until some hours later except in the submerged cut ends of the stems. The presence of an inner film of fatty substance is presumably primarily responsible for the delay in the lateral transport of the aqueous dye.

Suberization of the other elements of secondary xylem—fibers, parenchyma, and rays—resembles that seen in the leaf and stem cells already described and, as in these, becomes more marked toward the end of the growing season.

Suberization of the pith may be striking, as in *Platanus* (figs. 28–30). The central cells, larger than the marginal, are approximately isodiametric with heavily pitted cell walls. They are fairly closely packed, and the intercellular spaces are comparatively small. The protoplasts of the cells die fairly early, at about the time cambial activity begins. Chromic acid is particularly effective in the study of this tissue. On irrigation with the acid, all cell walls swell considerably. If suberization is incomplete, the middle lamella disintegrates as in maceration, but on both sides of it thin suberin films remain outlining the cells. If suberization is heavy, the middle lamella is completely impregnated, and at the end of the reaction a composite network persists. In both cases the intracellular lamella, a papillate sphere in which the papillae correspond to the pits, is isolated within the cell.

GYMNOSPERM.—Internal suberization of gymnosperm tissues is essentially similar to that of angiosperms. In the characteristic xylem of *Pinus* (4) growth rings are clearly defined; the tracheids range from 35 to 60 μ in diameter and have bordered pits on the radial walls. Rays are 1-seriate, or 3–5-seriate if inclosing a transverse resin canal, and consist of prominently dentate ray tracheids

FIGS. 7–12.—Figs. 7–9, *Platanus racemosa*, paradermal median sections of leaves. Fig. 7, from soft green leaf about one-third grown; young branched mesophyll cells and intercellular spaces, and intercellular lamella; stained with eosin. Fig. 8, from mature but still unhardened leaf; fully differentiated branched mesophyll cells; stained with IKI. Fig. 9, from half-grown leaf, during irrigation with IKI-H_2SO_4, showing intercellular suberin lamella and swollen cellulose wall. Figs. 10–12, from mature leaves and stem during IKI-H_2SO_4 irrigation. Fig. 10, *Persea americana*, leaf, paradermal median section, showing intercellular suberin lamella and swollen cellulose wall. Fig. 11, *Thamnosoma montana*, stem, transverse section, showing intercellular suberin lamella and swollen cellulose wall of palisade parenchyma cells. Fig. 12, *Encelia farinosa*, leaf, showing intercellular suberin lamella and swollen cellulose wall of palisade parenchyma cells.



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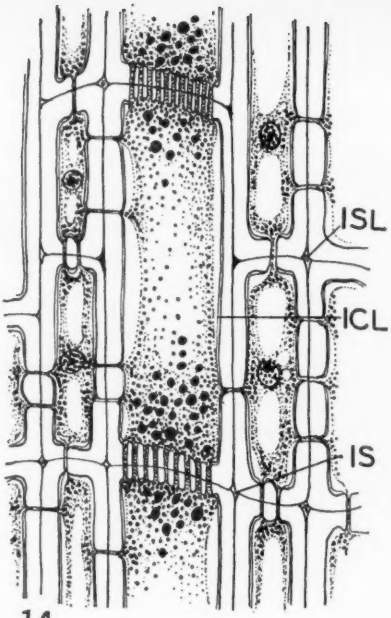

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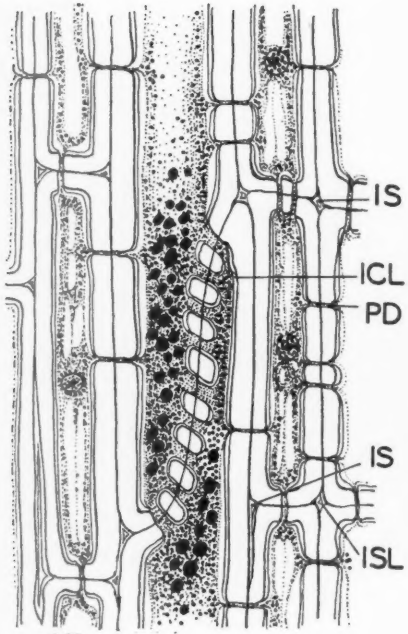

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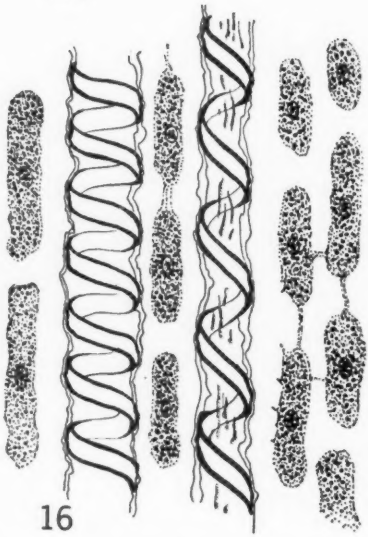

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FIGS. 13–16.—Sections from internodes of *Ricinus communis* during (figs. 13–15) and after (fig. 16) IKI-H_2SO_4 irrigation. *ICL*, intracellular lamella; *ISL*, intercellular space lamella; *IS*, intercellular space; *PD*, plasmodesmata. Fig. 13, primary internode, tangential section through cortical chlorenchyma, showing intercellular and intracellular suberin membranes. Fig. 14, primary internode, longisection, showing sieve tube and adjacent cells. Fig. 15, secondary internode, showing sieve tube and adjacent cells. Fig. 16, primary internode, longisection, showing two spiral vessels after treatment with IKI-H_2SO_4 and disappearance of cellulose.

and thicker walled ray parenchyma. Internal suberization is clearly evident in tracheids and in rays, and, as in angiosperms, lamellae increase in thickness toward the end of the growing season (figs. 31–33, 36).

Root

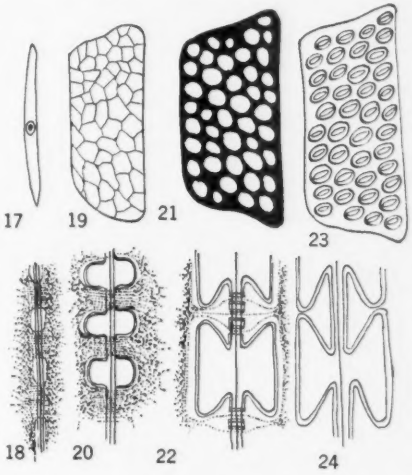
In order to complete the survey of the tissues of the axis, the roots of seedlings of various ages and of mature plants were examined. Vigorous seedlings of *Vicia faba* and of *Ricinus communis*, grown in well-watered, well-drained soil under optimum field conditions, ranged in height from 6 to 30 cm. As in the stem, intercellular spaces differentiate in the cortex behind the apical meristem, and in these there appear the first traces of suberization. Later the intracellular suberin lamellae become evident. In brief, parenchyma and vascular tissues are similar in stem and root as regards suberization.

One surprising observation was made in the root-hair zone of both *Vicia* and *Ricinus* seedlings: a thin layer of fatty material similar in behavior to cutin and suberin is present on the *external* surface of all root hairs. This film is indestructible in IKI-H_2SO_4 (80%) (figs. 37–39). It occurs on all living root hairs from papillae to full-length cells and is slightly thicker on the full-grown ones than on the younger. Since the root-hair zone is the principal region of absorption of water and salts (10), it is evident that the physical properties, such as impermeability, usually attributed to suberin require some reconsideration.

Flower

Since suberin pellicles were found in all vegetative organs, it seemed probable that they would also occur in flowers. It also appeared possible that in petals of long-lasting flowers, such as orchids, the internal lining might resemble that of leaves in thickness and visibility. In *Cymbidium* and *Epidendrum* this idea proved to be correct.

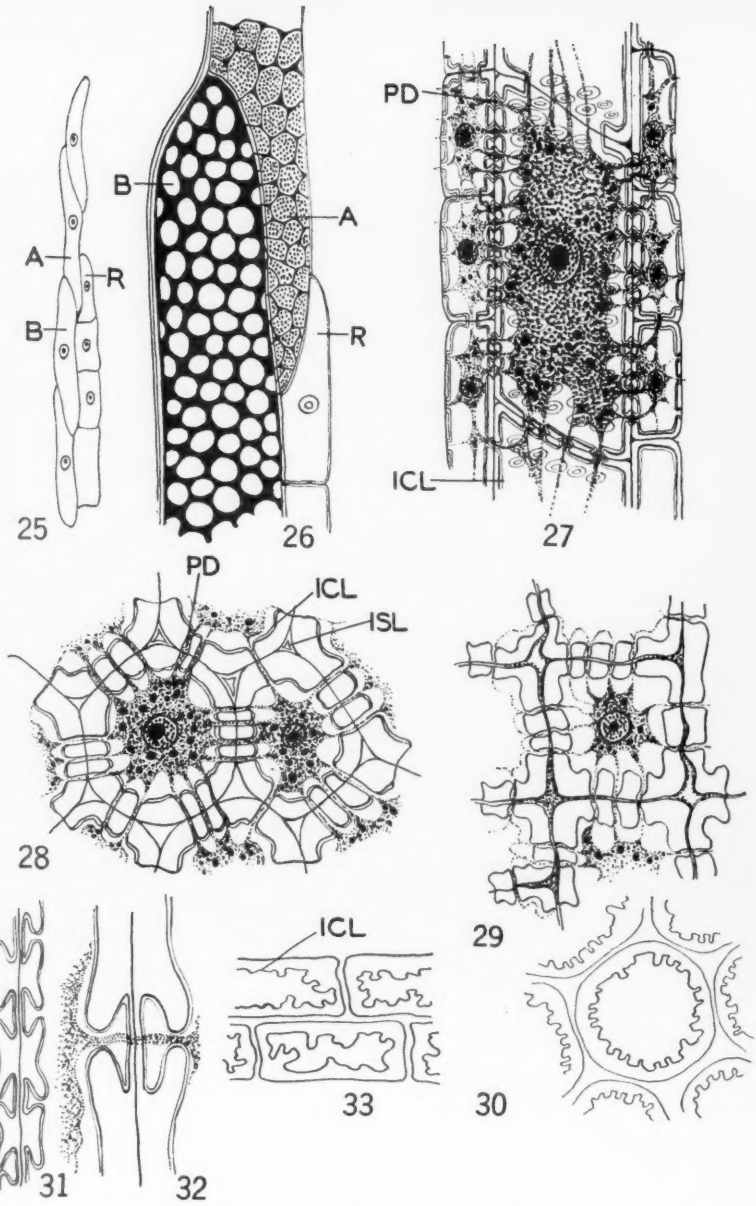
In the fully opened flower of *Cymbidium* the cuticle of both upper and lower epidermis of petals is about 1 μ thick. In surface view it is marked by larger (± 3 μ) and smaller (± 1 μ) darker disks, plugs of cutin-like material which block the exit of the plasmodesmatal canals.



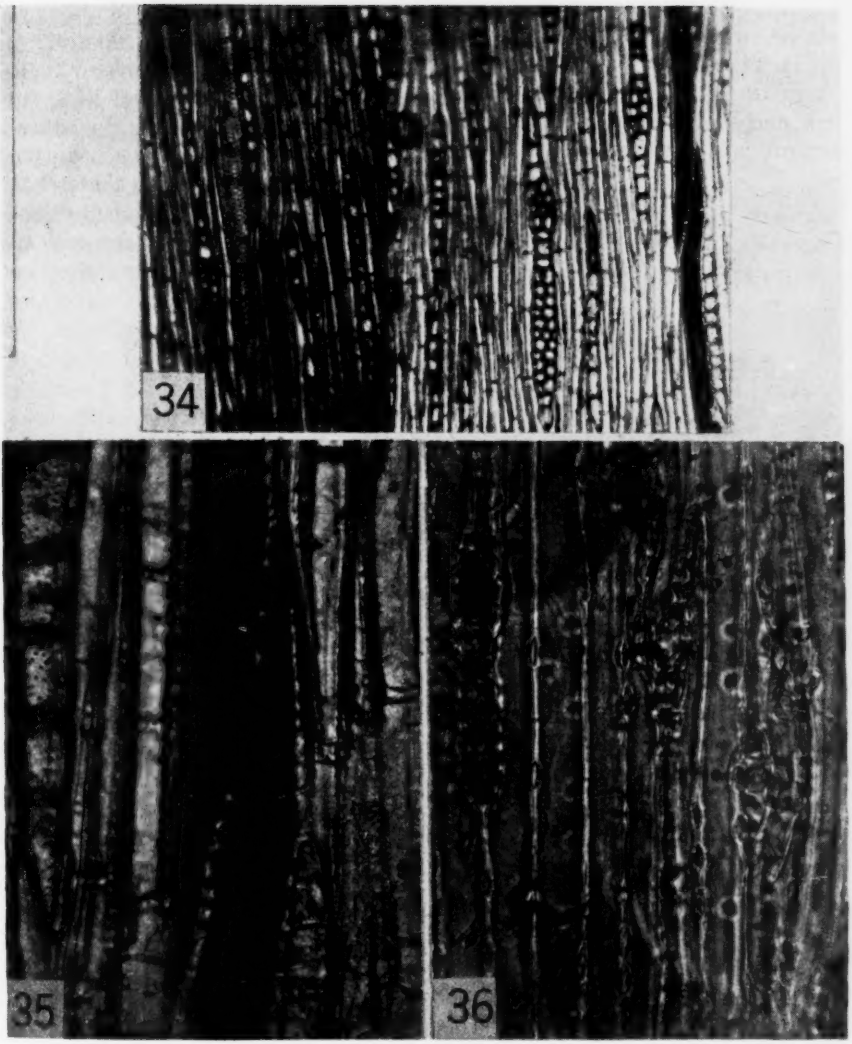
Figs. 17–24.—*Platanus racemosa*, diagrams of differentiation of border-pitted vessel segment and appearance of suberin lamella.

Upper and lower subepidermal layers are relatively compact with small intercellular spaces. The central parenchyma, 3–5 cells in depth, consists of 6–8-armed cells similar to those in the mesophyll of a typical leaf (figs. 40–45).

Internal suberization is similar to that seen in leaves. The intercellular spaces are lined with a thin but perfectly distinct pellicle, and, intracellularly, a film of the same type though more tenuous is also evident. Both films react as do those in young leaves. They are translucent



FIGS. 25–33.—Figs. 25–27, *Ricinus communis*, old stem, longisection during secondary thickening showing differentiation of vessel segment. *A*, *B*, vessel-segment initials; *ICL*, intracellular lamella; *ISL*, intercellular space lamella; *PD*, plasmodesmata; *R*, ray (camera lucida). Figs. 28–30, *Platanus racemosa*, current shoot, from transections (figs. 28, 30) and longisection (fig. 29) showing pith during IKI-H_2SO_4 irrigation. Figs. 31–33, *Pinus ponderosa*, three-year twig, from longisections (figs. 31, 32) during IKI-H_2SO_4 irrigation showing tracheid wall and from radial section (fig. 33) after irrigation, showing ray cells.



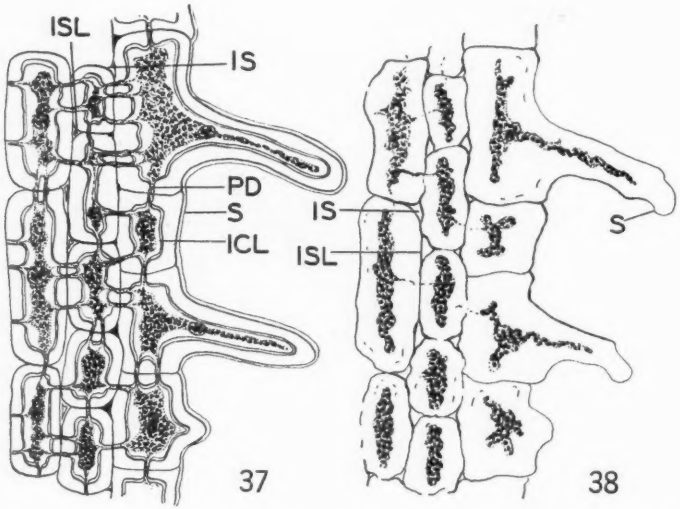
FIGS. 34–36.—Figs. 34, 35, *Juglans californica*, three-year twig cut under eosin–India ink aqueous solution; fig. 34, longisection showing India ink in vessel lumen, surface of bordered-pitted vessel, rays; fig. 35, detail of figure 34, junction between two vessel segments showing intracellular lamella. Fig. 36, *Pinus coulteri*, three-year twig, longisection, showing intracellular lamella within tracheids.

when mounted in water or glycerin and stain yellow in IKI. Following irrigation with H_2SO_4, as the cellulose walls swell and disintegrate, these films darken, become finely puckered, and eventually fragment into fine indestructible granules.

In contrast to orchids the petals of pelargoniums last for 1 or 2 days only before wilting. The conical cells of the epidermis of *Pelargonium* are cutinized, the cuticle not markedly different in thickness from that of the orchid. The walls of the branched mesophyll cells are very thin, but intercellular and intracellular films may still be distinguished, although, in contrast to the orchid, they are at the limit of visibility. It is therefore possible that the keeping quality of flowers may depend, at least in part, on internal suberization.

Fruit

The unit of structure of the fruit, the carpel, more or less resembles a typical leaf in structure. The pod of *Vicia faba*, covered by a firmly cutinized epidermis, consists in the main of branched parenchyma elements similar to those of leaf mesophyll. As in the leaf, suberization of intercellular spaces and of the inner surface of the cell wall occurs throughout.



FIGS. 37, 38.—*Vicia faba*, seedling, longisections of root-hair zone. Fig. 37, during IKI-H_2SO_4 irrigation, showing root hairs with external pellicle. Fig. 38, after IKI-H_2SO_4 irrigation, showing persistence of external pellicle, and intercellular lamellae.

Discussion

The cell wall has been the subject of a vast number of detailed physical and chemical investigations (**1, 3, 6, 7, 11, 12, 13, 21**), and it is recognized that, today, microscopic observation and microchemical techniques are limited in their possibilities of adding significantly to the knowledge of fundamental structure. However, the membranes described in this paper, and hitherto neglected, are

always clearly visible in fresh mature tissues under oil immersion; they are also generally identificable under lower magnifications (440×, 100×). It is only with great difficulty that they are recognized in the shrunken walls of sections of imbedded material stained by current methods. The first traces of deposition are, however, beyond the limits of visibility of the ordinary microscope. Doubtless, in the not-too-distant future, with the aid of the electron microscope, it will be possible to complete the story.

When I first reported on the internal suberization of the citrus leaf (**20**), I was not aware of any previous investigation of the general problem. The comparatively recent papers of ARZT (**2**) and HÄUSERMANN (**8**) have apparently escaped the notice of other botanists also; otherwise the occurrence of an "inner cuticle" could hardly have failed to be discussed somewhere in physiological textbooks in relation to permeability, fat metabolism, respiration, transport of solutes, and transpiration. If such discussion exists, I am unfortunately unaware of it. The present study has shown that a suberin-like material is deposited not only on the internal surface (**22, 24**) of leaves but also throughout the whole system of intercellular spaces of stem, root, flower, and fruit. In addition, within each cell, lining the cell wall adjacent to, but distinct from, the plasma membrane, there exists a very thin film of material similar optically and, so far as determined, microchemically to the lamella of the intercellular spaces.

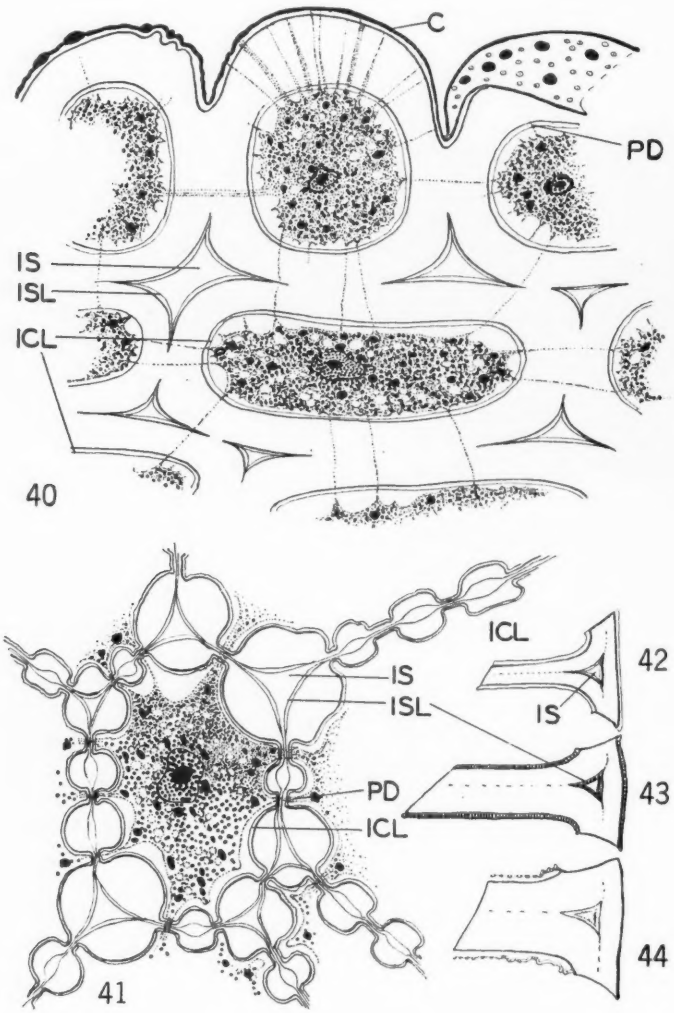
A somewhat surprising result was obtained during the examination of the root-hair zone. The outer surface of young developing root hairs is covered by a fatty film resistant to strong sulfuric acid and apparently similar to the intercellular and intracellular films already described.

The chemical precursors of suberin are presumably laid down in very young cells as monomolecular layers on the inner face of the cell wall by the activity of the plasma membrane. These materials can pass through the cell wall of the expanding cells and be deposited on the surface of the differentiating intercellular spaces. On contact with air, these—as stressed by PRIESTLEY in his discussion of cork

FIG. 39.—*Vicia faba*, photomicrograph of section similar to figs. 37, 38.

formation (15)—eventually form a film. The films in young cells are extremely thin but later thicken to a greater or lesser extent. One aspect of the aging of cells may therefore be considered as progressive suberization resulting from a gradual intensification of fat metabolism.

The hardness of the leaves of desert, chaparral, or alpine plants is accounted for not only by surface cuticularization



FIGS. 40–44.—*Cymbidium* hybrid, petal, during IKI-H_2OS_4 irrigation. *C*, cuticle; *ICL*, intracellular suberin lamella; *ISL*, intercellular space lamella; *IS*, intercellular space; *PD*, plasmodesmata. Fig. 40, transection, showing epidermis and subepidermis. Fig. 41, paradermal median section, showing typical branched parenchyma cell. Figs. 42–44, diagrams of optical sections of part of cell wall during IKI-H_2SO_4 irrigation, showing continuous, beaded, and fragmenting condition of intracellular lamella.

but also by internal hardening. The relative value of such causal factors (**15**) as drought, temperature, and sunlight on suberin formation awaits ecological investigation.

Internal suberization of xylem vessels must necessarily be taken into account in any future discussion of transport of solutes. The presence of a relatively impervious membrane may be an important factor in aiding in the building-up of negative pressures during active transpiration. At the same time it may also be responsible for retardation of the lateral transport of aqueous solutions. The physical nature of these membranes, however, requires thorough reinvestigation in relation to their actual permeability to fluids and to gases.

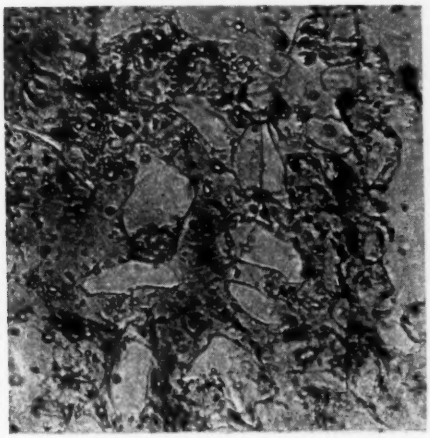


FIG. 45.—*Epidendrum* sp., paradermal section of petal, showing typical branched mesophyll cells; stained with IKI.

Since the micropitted appearance of the intracellular membrane is observed in vessels freshly mounted in water, it cannot be considered an artifact. It is therefore evident that the surface area of the protoplast is vastly increased by the innumerable corresponding micropapillae of the plasma membrane. Indications of a similar condition are occasionally observed in other cells but are less readily detected than in the xylem elements. It may well be, however, that the cell surface is not completely smooth as pictured hitherto and that, from the minute projections here described, there extend into the cell wall microplasmodesmata detectable by the electron microscope.

Summary

1. Suberin is used in this paper as a general term to connote a varnish-like wall material resistant to strong sulfuric and strong chromic acids. The most useful technique for identification is the standard microchemical test for cellulose, staining with IKI, followed by irrigation with H_2SO_4 (under continuous observation).

2. The internal surface, i.e., the entire system of intercellular spaces in leaf, stem, root, flower, and fruit, is lined by a suberin lamella. The inner surface of all cell walls is also lined by a more tenuous pellicle of apparently similar material which is distinct from the plasma membrane.

3. The degree of internal suberization varies in xerophytes, mesophytes, and hydrophytes and in tissues of different age. In young leaves suberin films are thin; they stain readily with IKI, darken, wrinkle, and eventually fragment on irrigation with H_2SO_4. The particles persist, however, as a tidemark of indestructible fragments after the complete disintegration of the cellulose walls. In old and hard leaves of various types, suberin impregnates the entire cell wall including the middle lamella. On irrigation with H_2SO_4, wall swelling is negligible, and the tissue emerges practically unchanged in structure.

4. Suberin films are first detectable in

expanding tissues when intercellular spaces begin to appear. In mature cells they measure $\pm 1\ \mu$ in thickness. The aging of cells is thus accompanied by progressive suberization resulting from an intensification of fat metabolism.

5. The external surface of developing root hairs in *Vicia faba* and in *Ricinus communis* is coated with a varnish-like film resistant to H_2SO_4 and in other respects indistinguishable from the intercellular and intracellular films of the root cortex and tissues of the stem and leaves of the same plants.

6. The inner surface of the vessel segments, and probably also of other cells, is not smooth as generally depicted but is micropitted, and the surface of the protoplast is correspondingly micropapillate. It is possible that the electron microscope may demonstrate that micro-plasmodesmata extend from the micropapillae into the cell wall.

7. The significance of internal suberization must necessarily be considered in future physiological discussions of such problems as transport of solutes, permeability, respiration, fat metabolism, and transpiration.

I am indebted to Miss ELIZABETH BAKER for the photomicrographs in this paper.

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COMPARATIVE HISTOLOGY OF NATURALLY PARTHENOCARPIC, HORMONE-INDUCED PARTHENOCARPIC, AND CAPRIFIED FIG SYCONIA

MESUT BASKAYA AND JULIAN C. CRANE[1]

Introduction

The edible fig is a dioecious plant, and pollination (caprification) is effected through the agency of a small hymenopterous insect, *Blastophaga psenes* L.

The fig fruit, a syconium, is a fleshy, hollow receptacle, the inner wall of which, when immature, is lined with flowers and, upon maturity, by numerous fruitlets (achenes). To avoid confusion between horticultural and botanical nomenclature, the receptacle or horticultural fruit is termed "syconium" in this paper. It is characterized by having at its distal end an orifice or ostiole which connects the cavity of the syconium with the exterior and which is superficially closed by a series of overlapping bracts. The *Blastophaga* passes through this opening at the time of caprification.

Of the four most important figs produced in California, the syconia of the Adriatic, Mission, and Kadota varieties, belonging to the common type (7), are completely parthenocarpic. Syconia of these varieties contain only female flowers which do not require the stimuli of pollination and fertilization to make the figs set and mature. Although the flowers of most common figs are usually unpollinated, the inner layer of the ovary—the endocarp—sclerifies and imparts hardness to the so-called "seed." The mature syconia, therefore, contain numerous achenes that are devoid of embryos or endosperm tissue. CONDIT (7) has used the term "cenocarpy" to denote the production of an achene that has a normally developed ovary wall but does not contain an embryo.

The Calimyrna fig variety exhibits the typical characteristic of the Smyrna type by being almost completely nonparthenocarpic. Syconia of this group set and mature only after the flowers have been pollinated and the ovules fertilized, with the resultant development of ovaries into achenes. Without the stimuli of pollination and fertilization, the immature figs cease growth and drop from the trees. For discussion of the problems associated with caprification of Calimyrna figs, the papers of CALDIS (3), CONDIT (5), SMITH (17), and SMITH and HANSEN (18) are suggested.

As pointed out above, caprification is not necessary for syconia to set and mature in figs classified as of the common type. When caprification does occur, however, rather marked changes take place in size, color of skin and pulp, texture, flavor, quality, and tendency for the syconia to split upon maturity. These effects are especially noticeable in the Kadota variety (6). The differences between caprified and parthenocarpic Kadota syconia, as noted by CONDIT (5), are as follows: "The color of the uncaprified fig is light, lemon yellow, the surface somewhat glossy; of the caprified fig, the color is green or yellowish green and the surface dull. Ribs on uncaprified figs

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practically absent, on caprified fruit ribbed appearance marked, especially in the wilted specimens. Meat or rind seemingly little affected in thickness and texture. Color of flesh in uncaprified figs amber or pinkish-amber to light strawberry, in caprified specimens much deeper. Uncaprified figs, practically seedless, at least seeds small and hardly noticeable; caprified figs with numerous, large, fertile seeds. Flavor of caprified figs sweeter and richer." No comparative histological examinations on caprified and parthenocarpic syconia of the same variety have been reported.

Calimyrna fig syconia can be induced to set parthenocarpically by application of an aqueous spray of gamma-(indole-3)-*n*-butyric acid (**9**). Reasons for attempting to eliminate the costly and haphazard cultural operation of caprification have been discussed (**9**). Subsequent experimentation by BLONDEAU and CRANE (**2**) has shown that application of an aqueous spray of para-chlorophenoxyacetic acid likewise induces Calimyrna fig syconia to set and mature parthenocarpically. Externally, the chemically induced parthenocarpic syconia were not markedly different from syconia that had been caprified, except that the former were somewhat flattened perpendicularly to the central axis; internally the most striking difference was the apparent absence of achenes in the chemically induced parthenocarpic figs, since there was no sclerification of the ovary walls. This was interesting, since, as discussed above, the endocarp in the individual achenes does develop and becomes sclerified in the naturally parthenocarpic Adriatic, Mission, and Kadota varieties.

The present histological investigation was therefore undertaken in order to study and compare the developmental phenomena occurring in naturally parthenocarpic, hormone-induced parthenocarpic, and caprified syconia.

Review of literature

Although the literature on parthenocarpy induced by application of growth-regulating substances is extensive, with but few exceptions there have been no detailed histological comparisons of developmental changes in induced parthenocarpic fruits and in those produced as a result of pollination and fertilization. GARDNER and KRAUS (**12**) were perhaps the first to make such a histological study. They reported that the growth and development of parthenocarpic American holly fruits, set by spraying the open blossoms with indoleacetic acid, paralleled the development of fruits that were pollinated. The endocarp enlarged and hardened at about the same rate in the two types, and microscopic examination revealed no outstanding differences in the vascular system, in either fruit or pedicel. The chief difference between the two types was the lack of development of embryo or endosperm in the ovules of the parthenocarpic fruits.

Since the fig and the strawberry are similar morphologically, in that an enlarged receptacle, supporting numerous tiny achenes, makes up the major portion of the horticultural fruit and since both these fruits can be set with growth substances, one would expect their ovarian tissues to develop similarly under the artificial stimulation. To what extent the development of the ovarian tissues in parthenocarpic strawberries is normal remains to be determined histologically. GARDNER and MARTH (**13**) stated that parthenocarpic strawberries set with indoleacetic acid produced "apparently normal achenes which, upon subsequent examination, proved to be devoid of embryos." SWARBRICK (**19**) reported that

each parthenocarpic fruit produced had some seeds (achenes) but that they were sparsely distributed, while HUNTER (14) observed the achenes to be smaller on parthenocarpic than on pollinated strawberries. On the other hand, ZIMMERMAN and HITCHCOCK (20), who induced the development of parthenocarpic strawberries by applying vapors of methyl naphthaleneacetate to unpollinated flowers, stated that the receptacles matured normally "but had few or no achenes."

BEAL (1) studied the development of the ovaries of *Lilium regale* following treatment with lanolin mixtures of indoleacetic, alpha-naphthaleneacetic, and beta-naphthoxyacetic acids alone and in combination. He reported very little difference in the histological responses to the various substances used. The increase in size of the ovaries following treatment resulted from the enlargement of cells composing the carpels and the outer integuments of the ovules. Certain regions of the carpel walls, the inner integuments, and the nucellus shrank and collapsed relatively early in development. None of the treated ovaries produced an embryo or endosperm tissue.

MANN (16) recently pointed out that if the generally observed response of ovary-wall development to growth substances follows, in stone fruits such as the peach, cherry, and plum, a pattern similar to that in the fruits mentioned above, then parthenocarpic fruits of those species would still contain a pit, as the pit in stone fruits is the endocarp or inner layer of the ovary wall. Although this is a logical conclusion, it might not necessarily hold true, since endocarp development in the achenes in fig syconia is shown later in this paper to be dependent upon the particular growth-regulating chemical used to induce parthenocarpy.

Material and methods

Naturally parthenocarpic and caprified syconia of the Kadota variety were used because, as previously mentioned, caprification affects the syconia of this variety more noticeably than it does in other varieties of common figs. In California, Kadota figs are used principally for canning. Since the green color which develops in the skin following caprification is unattractive in the fresh-fruit market and unacceptable to canners, the syconia are not caprified commercially but develop parthenocarpically. As the material used was collected in a commercial orchard and since caprified as well as parthenocarpic figs were desired for histological comparisons, a number of syconia were caprified by placing white muslin bags over a number of branches and introducing male or caprifigs containing *Blastophaga*. After the *Blastophaga* had emerged from the caprifigs and entered the Kadota syconia, the bags were removed from the branches.

Calimyrna fig samples were likewise collected in a commercial orchard that received the standard caprification procedure, which began on June 25 and ended on July 5, 1948 (fig. 1). This procedure consisted merely of suspending a certain number of caprifigs in each Calimyrna tree. Syconia of the Calimyrna variety develop normally until about 30 mm. in diameter, when, if not caprified, they shrivel and drop from the trees. To induce parthenocarpic development in this variety by the application of growth substances, it was necessary, therefore, to exclude the pollen-carrying *Blastophaga* by covering the test branches with muslin bags from June 24 until July 12.

Several growth substances were applied to the leaves and young syconia; the details regarding methods, concen-

trations, time of application, and responses were reported previously (2). For this histological investigation, parthenocarpic syconia induced by the application of 600 p.p.m. of gamma-(indole-3)-*n*-butyric acid and 60 p.p.m. of parachlorophenoxyacetic acid were used. These chemicals hereafter will be referred to as "IBA" and "PCPA," respectively.

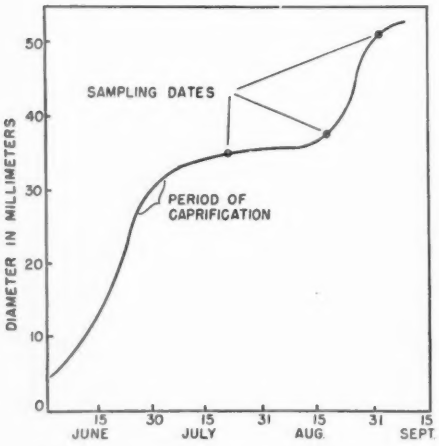


FIG. 1.—Typical growth curve of either induced parthenocarpic or caprified Calimyrna fig syconia, with period of caprification and dates of sampling indicated.

In order to determine the average size of a particular type of cell or tissue, at least 15, and in most instances from 100 to 200, individual readings were recorded. Measurements of cells composing the various tissues in the receptacle are based on their appearance in radial sections of the syconia. Measurements of various ovarian and seed tissues, on the other hand, are based on the appearance in longitudinal sections of the achenes. In both cases, "width" refers to the distance from the outer to the inner tangential walls of a particular cell (that is, the radial dimension), while "length" refers to the distance from one radial wall to the opposite (that is, the tangential dimension).

All cell and tissue measurements of the various parts of parthenocarpic syconia induced with PCPA were similar to measurements obtained on parthenocarpic syconia induced with IBA, with the exception of the endocarp. The data in the accompanying tables, therefore, apply only to syconia induced with IBA. Differences in endocarp development will be discussed later.

In figure 1 the three dates of sampling are shown in relation to growth in diameter of Calimyrna fig syconia; the curve is typical for either caprified or parthenocarpically induced figs. The dates of sampling were the same for the Kadota variety, and the relationship of sampling date to fruit development was practically identical with that of the Calimyrna variety. The samples consisted of five syconia of each variety or treatment. Syconia collected on a particular date were all of the same age, that is, only figs borne at the most proximal nodes on wood of the current season were used.

A square-centimeter segment through one side of each syconium was removed from a standard position toward the distal end. The material was killed and fixed in formalin-acetic-alcohol, prepared according to the usual paraffin method, sectioned perpendicularly to the epidermis at 10 μ, and stained in safranin and fast green.

Phloroglucin and hydrochloric acid were used for the detection of sclerified and lignified cells. Potassium iodide and sulfuric acid were used to test for the presence of cellulose in the collenchyma tissue in Kadota fig syconia. An 85% sulfuric acid solution was employed for the detection of calcium oxalate crystals in the various tissues mentioned later. The

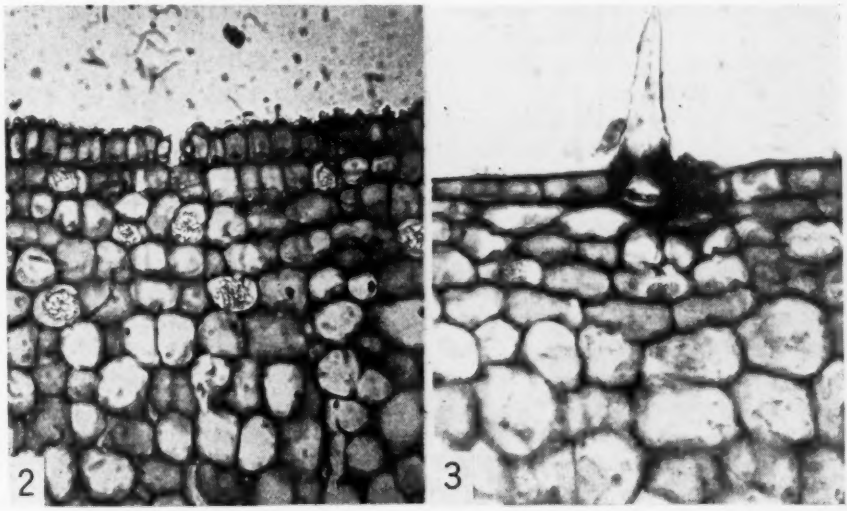
presence of cutin and pectin was determined by the use of Sudan IV and ruthenium red, respectively.

Observations

Syconium

Epidermis.—When sampled on July 22, the external epidermal cells of both the induced parthenocarpic and the caprified Calimyrna figs were rectangular in radial section, with the long axis perpendicular to the outer surface of the syconium (fig. 2). This condition persisted in caprified syconia to maturation. On the other hand, external epidermal cells of the induced parthenocarpic figs gradually changed as the syconium enlarged until, at maturity, these cells were rectangular in radial section, with the long axis parallel to the surface (fig. 3, table 1). In both parthenocarpic and caprified Kadota fig syconia, the external epidermal cells varied in radial section from square to rectangular, with the long axis of the rectangular cells parallel to the surface on each sampling date. No significant differences in sectional area of epidermal cells were noted between parthenocarpic and caprified syconia of either variety.

A thin layer of cuticle was found on the outer walls of the external epidermal cells of the syconium, as well as on the epidermal cells lining its interior surface. The pedicel and perianth of the individual flowers, the outer ovary wall, and the integuments were likewise covered by cuticle. The average thickness of the cuticle on the external epidermis was greater in caprified than in induced parthenocarpic figs of both varieties (table 1). The cuticle on the inner epidermis, pedicel and perianth of the flowers,



Figs. 2, 3.—Cross sections through epidermis and cortex of induced parthenocarpic Calimyrna fig syconia. ×384. Fig. 2, immature; shape of epidermal cells in caprified Calimyrna syconia was similar to above on all three sampling dates. Note calcium oxalate druses in outer five or six layers of parenchyma cells in cortex. Fig. 3, mature; epidermal cells with long axes parallel to surface of syconium. Compare with fig 2.

TABLE 1

AVERAGE MEASUREMENTS IN MICRONS OF CELLS COMPOSING EXTERNAL EPIDERMIS AND CORTEX OF SYCONIUM AND OVARY WALL OF ACHENES IN FIG SYCONIA

KIND OF SYCONIA AND DATE OF SAMPLING	EPIDERMIS					CORTEX								OVARY WALL					
	Epidermal cells		Cuticle			Collenchyma				Parenchyma				Exocarp		Mesocarp			Endocarp diameter
								Wall thickness		Outer		Inner				Outer		Inner diameter	
	Length	Width	Max.	Min.	Av.	Length	Width	Tangential	Radial	Length	Width	Length	Width	Length	Width	Length	Width		
Calimyrna, induced parthenocarpic:																			
July 22	9.4	15.2				No collenchyma				16.1	15.9	29.6	31.3	17.5	73.3	18.9	5.0	3.1	None
Aug. 19	11.0	13.8								16.2	17.8	34.3	33.4	23.5	93.5	15.4	2.5	3.1	None
Sept. 2	15.4	10.0	5.8	2.4	4.1					25.1	19.4	34.5	45.9	20.6	63.2	14.1	3.0	3.1	None
Calimyrna, caprified:																			
July 22	9.9	15.9				No collenchyma				19.8	15.3	31.7	28.7	23.1	76.8	16.2	7.5	4.5	19.5
Aug. 19	10.9	19.3								19.9	16.4	33.8	30.8	25.5	91.6	14.1	8.0	6.2	17.7
Sept. 2	12.9	17.9	8.4	3.4	5.9					21.9	16.8	32.1	31.1	28.2	98.0	15.2	8.0	6.2	17.8
Kadota, naturally parthenocarpic:																			
July 22	13.1	11.1				29.3	11.8	1.8	0.9	No		24.2	25.5	27.1	103.3	10.6	6.0	6.2	28.0
Aug. 19	15.3	13.3				34.5	11.4	1.5	0.8	outer		29.5	33.2	27.4	89.1	13.2	6.2	6.2	30.0
Sept. 2	16.9	9.4	5.0	3.1	4.5	35.2	10.3	0.9	0.8	parenchyma		30.9	34.1	25.6	82.5	Crushed		9.3	21.0
Kadota, caprified:																			
July 22	14.3	12.9				33.9	9.1	1.9	0.6	No		27.3	24.1	30.4	115.2	17.2	3.3	6.3	26.6
Aug. 19	15.5	11.4				35.5	13.2	1.1	0.8	outer		29.1	33.6	30.5	116.0	18.0	3.3	8.2	26.3
Sept. 2	19.7	6.2	7.0	2.8	4.9	40.3	13.6	1.1	0.8	parenchyma		38.3	28.2	Crushed		Crushed		10.3	21.3

outer ovary wall, and integuments in parthenocarpic syconia of both varieties was about 1.5 μ thick, as compared with about 2 μ in caprified syconia.

PARENCHYMA.—Parenchyma tissue forms the bulk of the receptacular portion of the syconium and is primarily storage tissue. The cells of the outermost five or six cell layers in the Calimyrna variety are thin-walled and closely adjacent to one another, with little or no intercellular space. These cells are generally isodiametric, but occasionally some are elongated tangentially. Numerous druses of calcium oxalate are found in this region. Cells of the inner parenchyma show great variation in size and shape and are partially separated by intercellular spaces. This type of tissue gives way to two or three layers of closely arranged, isodiametric cells adjacent to the inner epidermal layer that lines the cavity of the receptacle. The cells in this internal dense region are very similar to the five or six external layers. Calcium oxalate druses are abundant in this region also.

The individual parenchyma cells generally increased progressively in length and width from the first to the last sampling date (table 1). At maturity, the sectional area of the individual parenchyma cells in induced parthenocarpic Calimyrna and naturally parthenocarpic Kadota syconia was greater than that of similar cells in caprified syconia in both varieties (tables 1, 2).

The thickness of the parenchymatous tissue either decreased or remained about the same from the first to the last sampling date (table 2). Macroscopic examination on each date of sampling revealed, however, that there is an actual decrease in thickness of the parenchymatous area with development of an individual syconium to maturity: the cells in the inner parenchyma separate, the walls break down, and the tissue becomes thinner as a result of the expansion of the syconium, the outward pressure against the tissue by the numerous developing achenes, and the engorgement of the fruit pedicels with stored sugars. It is significant, however, that at each sampling the total thickness of the parenchyma tissue and the number of cell layers contained in it were greater in most cases in the parthenocarpic syconia than in the caprified syconia of both varieties (table 2).

COLLENCHYMA.—A detailed study involving microchemical tests and morphological and ontogenetic observations showed an average of six to seven cell layers directly beneath the external epidermis of both caprified and parthenocarpic Kadota figs to be collenchyma rather than parenchyma (fig. 4). Ergastic substances, primarily calcium oxalate druses, were found in the collenchyma tissue, corresponding to the presence of these materials in the outer parenchyma cells of the Calimyrna syconia.

The tangential walls of the collenchyma cells became progressively thinner as the syconium developed to maturity (table 1). The size of the individual collenchyma cell, as measured by length and width (table 1), as well as the total thickness of this tissue (table 2), was somewhat greater at maturity in caprified than in parthenocarpic Kadota syconia.

Although Kadota syconia contained a few more laticiferous tubes than Calimyrna syconia, no differences in the size of these tubes existed between parthenocarpic and caprified figs.

The size and number of the component cells in the vascular bundles were not markedly different in the various syconia examined.

ACHENE

The ovary wall of an achene in a naturally parthenocarpic syconium, as well as in one that has been caprified, consists of four distinct layers: the outermost, composing the exocarp, the next two, making up the mesocarp, and the fourth or innermost being the endocarp (fig. 5).

EXOCARP.—This tissue is a single layer of cells that are elongated perpendicularly to the surface of the ovary. Based upon the sectional area of the individual cells (table 1), the exocarp had developed rather completely in all specimens at the time of first sampling. In achenes of caprified Kadota syconia it was observed to be crushed on the last sampling date. It is clear that the exocarp cells on each date of sampling were, in most cases, longer and wider in the caprified than in the parthenocarpic syconia of both varieties (table 1).

TABLE 2

AVERAGE MEASUREMENTS IN MICRONS AND NUMBER OF CELL LAYERS IN TISSUES OF EPIDERMAL AND CORTICAL REGIONS IN FIG SYCONIA

KIND OF SYCONIA AND DATE OF SAMPLING	EPIDERMIS	CORTEX								
		Collenchyma			Parenchyma					
							No. of cells in 32,400 sq. μ		From epidermis to separation zone	
	Thickness	Thickness	No. of cell layers	No. of cells in 32,400 sq. μ	Thickness	No. of cell layers	Outer	Inner	No. of cell layers	Width
Calimyrna, induced parthenocarpic:										
July 22	15.2	No collenchyma			4593	152	80	33	40	1053
Aug. 19	13.8				3705	135	73	17	38	1300
Sept. 2	10.0				5119	156	54	14	38	1460
Calimyrna, caprified:										
July 22	15.9	No collenchyma			4116	139	92	19	45	1466
Aug. 19	19.3				3477	135	87	23	39	910
Sept. 2	17.9				2971	121	81	23	30	510
Kadota, naturally parthenocarpic:										
July 22	11.1	69.5	6	90	6169	182	90	23	52	1305
Aug. 19	13.3	79.6	7	69	4745	154	69	14	46	1300
Sept. 2	9.4	71.9	7	67	5451	162	67	14	46	1300
Kadota, caprified:										
July 22	12.9	54.1	6	76	4766	143	77	18	39	910
Aug. 19	11.4	92.6	7	71	4745	151	71	18	39	1040
Sept. 2	6.2	94.8	7	71	3284	115	71	16	49	1170

MESOCARP.—For convenience, the two layers of the mesocarp have been termed "outer" and "inner." The outer mesocarp was found to consist of a maximum of four layers of parenchymatous cells (table 3). The sectional area of the cells, as well as the thickness of the outer layers, was considerably smaller in parthenocarpic Calimyrna syconia than in

caprified syconia, whereas the converse was true in the Kadota variety. The significance of this difference will be discussed later.

The inner mesocarp consists of one or two layers of small cuboidal cells that are parenchymatous at the beginning of their ontogeny. Later, a single crystal of calcium oxalate is deposited within each, and the cell walls become sclerified. All samples collected on July 22 showed these cells in the parenchymatous condition, with crystals of similar size present in all syconia. Samples collected when the figs were mature (September 2) showed these cells to be completely sclerified in all syconia except those produced parthenocarpically with IBA or PCPA, in which no sclerification whatsoever took place, i.e., the inner mesocarp cells of both IBA- and PCPA-induced parthenocarpic syconia remained parenchymatous. The crystals of calcium oxalate in the inner mesocarp cells of mature parthenocarpic Calimyrna syconia induced with IBA were considerably smaller than crystals in the corresponding cells in caprified syconia. Whereas each inner mesocarp cell in IBA-induced syconia, as well as in the naturally parthenocarpic and caprified syconia, contained one large crystal, only one-third of these cells in PCPA-induced syconia contained crystals. The inner mesocarp of both induced parthenocarpic Calimyrna and naturally parthenocarpic Kadota figs consisted of only one cell layer, while in caprified specimens of both varieties this tissue contained one or two layers, depending upon the specimen (table 3).

Cells of the inner mesocarp in caprified Calimyrna syconia were twice as large in radial diameter as were corresponding cells in induced parthenocarpic syconia (table 1). A similar relationship was true in the Kadota variety, but to a lesser degree. The development of the inner mesocarp, as measured by its thickness, was considerably less in the parthenocarpic than in the caprified syconia of the respective varieties (table 3).

ENDOCARP.—Early in the development of the inner ovary wall, the compo-

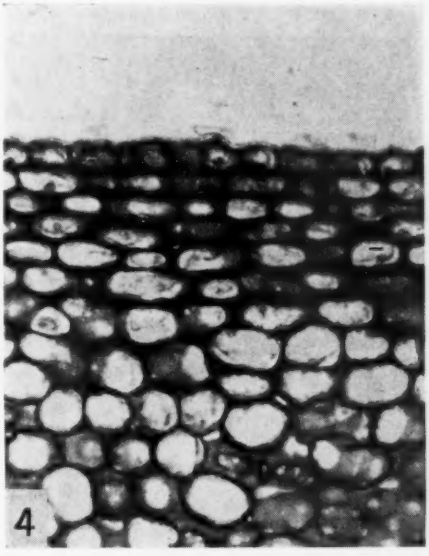


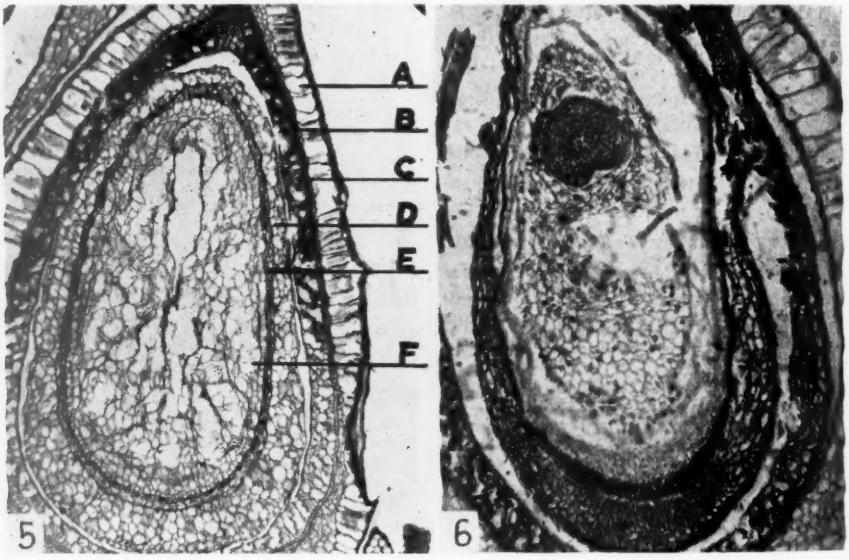
FIG. 4.—Longitudinal section through epidermis and cortex of mature caprified Kadota syconium. Note several cell layers of collenchyma tissue immediately beneath epidermis.

nent cells, of which there are from two to five layers, are ramiform in character but are thin-walled and parenchymatous. Later on, these cells are characterized by having a narrow lumen and very thick sclerified walls with distinct concentric layers. At the first sampling (July 22) the endocarp tissue in caprified Calimyrna, naturally parthenocarpic, and caprified Kadota syconia had become more or less completely sclerified (figs. 6, 7). Although endocarp tissue was present in PCPA-induced parthenocarpic

Calimyrna syconia, there was no evidence of sclerification (fig. 8). Except for a few cells at the apical and basal regions of the seed cavity, endocarp tissue was lacking in IBA-induced parthenocarpic Calimyrna syconia (fig. 9).

At maturity the same general picture was evident. In other words, although the endocarp tissue in caprified Calimyrna, naturally parthenocarpic, and caprified Kadota syconia had completely sclerified, in PCPA-induced parthenocarpic Calimyrna syconia sclerification was found to have occurred only in a single achene where there was a small area 40 μ in depth, 51 μ in width, and 410 μ in length (fig. 10). In IBA-induced parthenocarpic Calimyrna syconia, the endocarp was totally lacking (fig. 11). The few cells which had developed earlier at the apical and distal ends of the seed cavity had disappeared.

Although there was little difference in the diameter of endocarp cells between caprified and naturally parthenocarpic Kadota syconia (table 1), the thickness and number of cell layers in this tissue were somewhat greater in caprified than in parthenocarpic syconia (table 3). The cell diameter and total width of endocarp tissue in PCPA-induced parthenocarpic Calimyrna syconia were somewhat less than in caprified Calimyrna, naturally parthenocarpic, and caprified Kadota syconia. The width and number of cell layers composing the endocarp in caprified Calimyrna syconia were somewhat greater than in parthenocarpic and caprified Kadota syconia (table 3).



Figs. 5, 6.—Typical achene structure in caprified Calimyrna or caprified Kadota syconia. A, exocarp; *B*, mesocarp; *C*, endocarp; *D*, outer integument; *E*, inner integument; *F*, nucellus. ×96. Fig. 5, immature; sclerification of endocarp has begun as signified by dark area toward apex. Fig. 6, mature, showing partial development of embryo and endosperm.

OVULE.—The cells of the outer integu-

TABLE 3

AVERAGE THICKNESS IN MICRONS, NUMBER OF CELL LAYERS, AND OBSERVATIONS ON VARIOUS OVARIAN AND SEED TISSUES IN FIG SYCONIA

KIND OF SYCONIA AND DATE OF SAMPLING	OVARY WALL							SEED						
	Exocarp	Mesocarp				Endocarp		Outer integument		Inner integument		Nucellus	Endosperm	Embryo
		Outer		Inner										
	Thickness	Thickness	No. of cell layers	Thickness	No. of cell layers	Thickness	No. of cell layers	Thickness	No. of cell layers	Thickness	No. of cell layers			
Calimyrna, induced parthenocarpic:														
July 22....	73.3	15	3	3.1	1	None*	None*	15	1–2	6	1–2	Partially developed	None	None
Aug. 19....	93.5	5	2	3.1	1	None	None	15	1 Collapsed	9	1 Collapsed	Collapsed	None	None
Sept. 2....	63.2	3	1	3.1	1	None	None	5	1 Collapsed	2	1 Collapsed	Collapsed	None	None
Calimyrna, caprified:														
July 22....	76.8	30	4	6.5	1–2	78	4	46	2–3	32	3–4	Fully developed	Partially developed	Partially developed
Aug. 19....	91.6	16	2	9.3	1–2	71	4	3	1–2 Crushed	2	1 Crushed	Absorbed	Fully developed	Fully developed
Sept. 2....	98.0	16	2	9.4	1–2	89	5	2	1 Crushed	1	1 Crushed	Absorbed	Fully developed	Fully developed

* The few unsclerified cells at the apical and basal extremities of the seed cavity were not measured or counted.

TABLE 3—*Continued*

Kind of Syconia and Date of sampling	Ovary wall							Seed						
	Exocarp	Mesocarp				Endocarp		Outer integument		Inner integument		Nucellus	Endosperm	Embryo
	Thickness	Outer		Inner		Thickness	No. of cell layers	Thickness	No. of cell layers	Thickness	No. of cell layers			
		Thickness	No. of cell layers	Thickness	No. of cell layers									
Kadota, naturally parthenocarpic:														
July 22....	103.3	12	2	6.2	1	56	2	63	4	15	4	Fully developed	None	None
Aug. 19....	89.1	25	4	6.2	1	60	2	24	3 Collapsed	12	2 Collapsed	Collapsed	None	None
Sept. 2....	82.5	Crushed	Crushed	9.3	1	63	3	17	3 Collapsed	10	2 Collapsed	Collapsed	None	None
Kadota, caprified:														
July 22....	115.2	10	3	8.1	1–2	80	3	56	4	16	3	Fully developed	Partially developed	Partially developed
Aug. 19....	116.0	10	3	9.7	1–2	79	3	2	1 Crushed	2	1 Crushed	Fully developed	Fully developed	Fully developed
Sept. 2....	Crushed	Crushed	Crushed	13.4	1–2	64	3	1	1 Crushed	1	1 Crushed	Absorbed	Fully developed	Fully developed

ment are parenchymatous and irregular in shape. The cells of the outermost layer are elongate, with the long axis parallel to the surface of the achene, and are covered with a layer of cutin. In IBA- and PCPA-induced parthenocarpic Calimyrna syconia, as well as in all others examined, the maximum number of cell layers in the outer integument was observed at the first sampling. On the second sampling date, cell enlargement had taken place, but one layer of cells had also collapsed (table 3). This tissue had almost completely collapsed in the induced parthenocarpic syconia by September 2. The same general picture was observed in integument development in naturally parthenocarpic Kadota syconia, but here the original development was of a greater magnitude (table 3). The outer integument in caprified Calimyrna and Kadota syconia was crushed by August 19, probably as a result of embryo development.

In general, the inner integument in the various syconia examined followed the same pattern of development as did the outer integument (table 3).

Partial development of nucellar tissue was observed in the IBA- and PCPA-induced Calimyrna syconia sampled on July 22. By August 19, however, the nucellus in these syconia had completely collapsed, and only portions of cell walls remained. In contrast, the nucellar tissue in naturally parthenocarpic Kadota syconia had reached full development by the earlier date; but here, likewise, this tissue later collapsed. Nucellar development was complete by July 22 in both Calimyrna and Kadota caprified syconia. This tissue had been partially absorbed by the developing embryo by August 19 and was completely absent on September 2.

There was no endosperm or embryo development whatsoever in any of the parthenocarpic syconia. Growth of the embryo and endosperm in caprified syconia of both varieties had started on July 22. Embryo development was complete in some cases by August 19.

Discussion

An explanation for the change in shape of epidermal cells of induced parthenocarpic Calimyrna syconia as compared with caprified syconia, where no change in these cells was observed, is a point for speculation, particularly since similar changes were not observed in the Kadota variety. It is possible that the rate of epidermal cell division in induced parthenocarpic Calimyrna syconia was slower than cell division and enlargement in the cortical tissue, with the result that epidermal cells were stretched tangentially until at maturity they were rectangular in section, with their long axes parallel to the surface of the syconium. Similar changes need not occur in the Kadota variety, since the epidermal tissue here is underlain with collenchyma rather than with parenchyma, as in the Calimyrna variety.

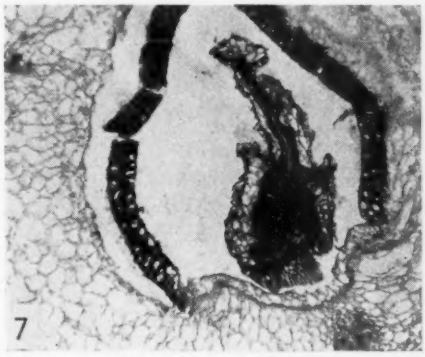
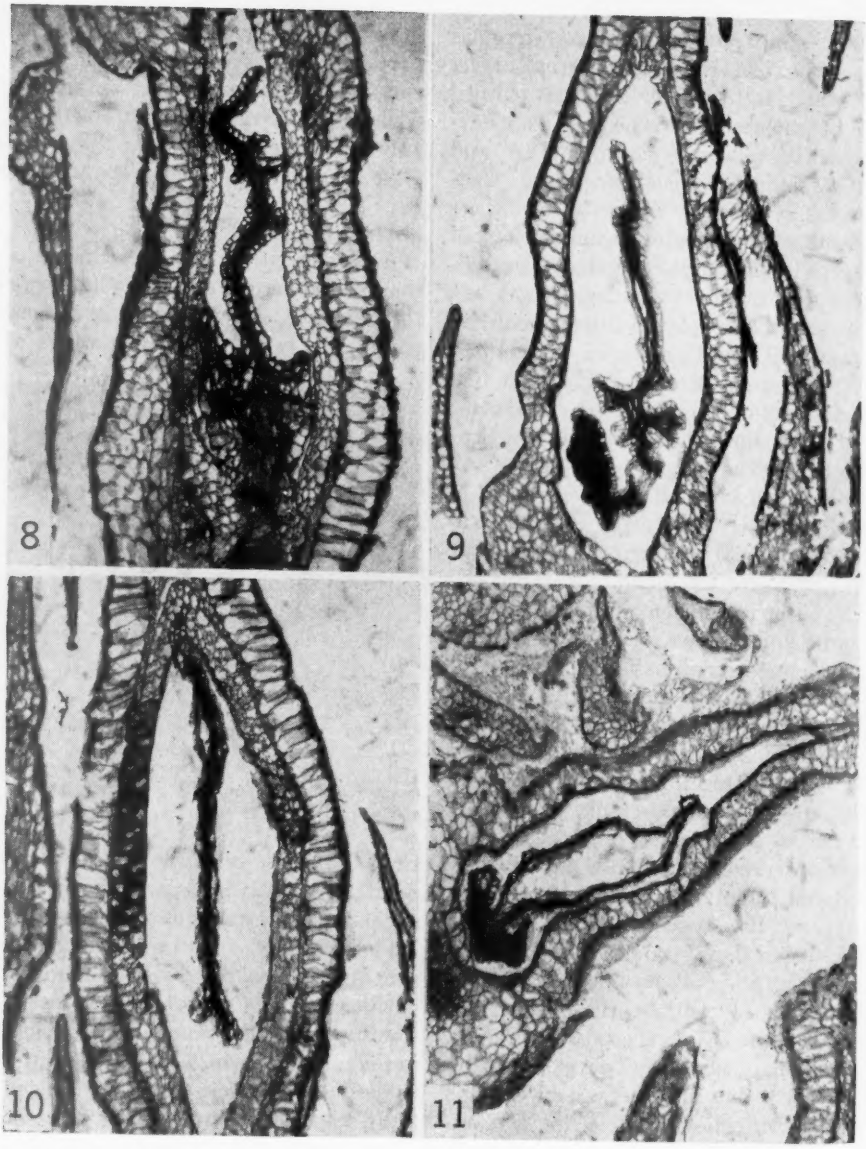


FIG. 7.—Typical achene in naturally parthenocarpic Kadota syconium with completely sclerified endocarp tissue.



FIGS. 8–11.—Typical achenes from parthenocarpic Calimyrna syconia induced by growth substances. ×96. Figs. 8, 10, induced by para-chlorophenoxyacetic acid; figs. 9, 11, by gamma-(indole-3)-*n*-butyric acid. Fig. 8, mature; integuments partially developed and nucellus collapsed; endocarp of ovary wall not sclerified. Fig. 9, immature; only evidence of endocarp development are few cells at apex and base of seed cavity. Fig. 10, mature; only achene examined which showed a partially sclerified endocarp—the dark area at left. Fig. 11, mature; complete absence of endocarp tissue.

As previously mentioned, the Kadota is the principal variety used for canning in California. In describing the syconia of this variety, CONDIT (6) stated: "The outer skin and white meat surrounding the pulp, constitute a sort of rind with a rubbery consistency, one of the characters which makes the fig suitable for commercial canning. This thick rind, however, is responsible for making an inferior, leathery product when dried." The present comparative histological study of parthenocarpic and caprified Kadota syconia indicates the anatomical basis for the superior qualities of this fig for canning purposes. The presence of the several layers of collenchyma cells beneath the epidermis appears to be responsible for the thick rind referred to above.

The producer of figs may be bothered by the splitting of the syconia during the period of final swell or about the time that ultimate size is reached. Although this is not a major problem in the Kadota variety, CONDIT (5) has reported that caprification of this variety increases the tendency for the syconia to split. Splitting of Calimyrna syconia, on the other hand, is a major problem. The amount of splitting varies from location to location, from season to season, and with the degree of caprification. The consensus is that excessive caprification promotes splitting, while lightly caprified syconia do not split. In other words, splitting of Calimyrna figs is apparently associated with the number of flowers that are pollinated within each syconium: the more flowers pollinated, the more severe the splitting.

CRANE and BLONDEAU (9) reported that induced parthenocarpic Calimyrna figs do not split. They found that the floral organs in these figs do not enlarge to the same degree as those in caprified figs, with the result that they only partially fill the cavity of the receptacle. They concluded that splitting may be the result of a growth phenomenon closely associated with the developing achenes in the syconium. The data obtained in the present investigation tend to corroborate this viewpoint: it was noted, for example, that development of the ovary wall, particularly of the exocarp and endocarp, was somewhat greater in both Kadota and Calimyrna caprified syconia than in parthenocarpic syconia of these varieties. Since an individual syconium contains several hundred developing fruitlets, the difference in volume increase of the ovary wall between parthenocarpic and caprified fruitlets would be sizable.

Splitting of syconia has also been observed to be more severe under conditions of high relative humidity. When the humidity is high and transpiration of the leaves is reduced, it is thought that the splitting results from an increased turgor pressure (4). It is logical, therefore, to conclude that splitting is the result of the growth of the fruitlets within the syconium, the degree of splitting being closely associated with certain environmental conditions.

Several studies of differences in chemical composition between parthenocarpic and seeded fruits have been reported. EWERT (11), working with a variety of pear which produced both parthenocarpic and seeded fruit, found that there was more sugar in the former than in the latter. JANES (15) stated that each region in red, ripe, parthenocarpic tomato fruits had a higher percentage of sugar than did corresponding regions of seeded fruits. CONDIT (5) published a list of analyses of caprified and parthenocarpic figs made by a number of workers, which showed that in several of the varie-

ties studied there was more sugar in parthenocarpic than in caprified syconia. BLONDEAU and CRANE (2), likewise, reported the sugar content of PCPA-induced parthenocarpic Calimyrna syconia to be 51% on a dry-weight basis as compared with 46% in caprified syconia.

The histological data reported here offer a partial explanation for the fact that parthenocarpic syconia contain a higher percentage of sugar than caprified syconia do. The cell size and total width of the parenchyma tissue in the cortex of both Kadota and Calimyrna parthenocarpic syconia were larger than in the corresponding caprified syconia. As the parenchyma of the cortex is primarily storage tissue and since the ratio of this tissue to the remainder of the syconium is larger in parthenocarpic than in caprified syconia, the difference in sugar content may be explained on this basis. Undoubtedly, as pointed out by JANES (15), the formation and development of seeds are directly associated with the chemical composition of other parts of the fruit. In seed development a certain amount of food is used which is drawn from neighboring tissues. In parthenocarpic fruits, however, the food which would normally be utilized for seed formation and development accumulates, resulting in a greater carbohydrate content than in pollinated fruits. The lack of competition for food materials between parenchyma tissue and developing achenes in parthenocarpic syconia might also help to explain the fact that parenchyma cell size and number of layers were greater in parthenocarpic than in caprified figs.

The responses in the development of the ovary wall when IBA or PCPA were used to induce parthenocarpy in Calimyrna syconia further demonstrate the high degree of specificity of synthetic growth substances and the diverse reactions they cause. BLONDEAU and CRANE (2) reported that three closely related compounds—PCPA, 2,4-dichlorophenoxyacetic acid, and 2,4,5-trichlorophenoxyacetic acid—when sprayed on young Calimyrna syconia, induced rather extreme responses. PCPA-induced parthenocarpic syconia developed and matured at the same rate as did caprified syconia; 2-4-dichlorophenoxyacetic acid, at the concentrations used, was ineffective in inducing parthenocarpy; and 2,4,5-trichlorophenoxyacetic acid promoted parthenocarpy, but the syconia matured 60 days in advance of syconia that were caprified. Evidence presented here shows that, although syconia induced parthenocarpically with PCPA or IBA were alike in most characteristics, an unsclerified endocarp was produced in the former case, and in the latter the endocarp was totally lacking at maturity. The inner mesocarp cells likewise failed to sclerify in both cases, and the content of calcium oxalate crystals in these cells was altered, particularly in the case of PCPA-induced parthenocarpic syconia.

It is interesting that measurements of cell size and of thickness of the various tissues composing the ovary wall are generally smaller in induced parthenocarpic Calimyrna than in naturally parthenocarpic Kadota syconia (tables 1, 3). Although this could be the result of varietal differences, the development of inner mesocarp and endocarp in parthenocarpic syconia induced with IBA or PCPA cannot be explained on this basis. The significance of these phenomena is not understood, but they are possibly the result of a difference in activity between the synthetic hormones applied to induce parthenocarpy and the natural hormone in the Kadota syconium. On

the other hand, the quantity of externally applied hormone might have been sufficient to induce parthenocarpy but insufficient to meet the total requirements for development of the ovary wall. Translocation of the externally applied hormone to regions where needed is another factor that must be given consideration in this connection. All these factors merely emphasize the necessity for further research in order that we may understand the diverse responses obtained through the use of synthetic hormones.

Data published by CRANE (**8**) and by CRANE and BLONDEAU (**10**) indicate that the cyclic growth of the fig syconium is due to variation in the supply or activity of a hormone produced within the syconium. As they pointed out, the growth-controlling hormone is not produced in the embryo, since the growth of both parthenocarpic and caprified syconia is cyclic in character—the three characteristic growth periods being of about the same duration in the two kinds of syconia. They believed the source of hormone to be the ovarian or receptacular tissue. It is known now that endocarp tissue, at least, does not contribute to the hormone supply in IBA-induced parthenocarpic Calimyrna syconia, in which it is lacking.

Summary

1. Histological comparisons have been made of the following kinds of fig syconia sampled three times during the growth period: (*a*) naturally parthenocarpic Kadota, (*b*) caprified Kadota, (*c*) parthenocarpic Calimyrna induced by parachlorophenoxyacetic acid (PCPA), (*d*) parthenocarpic Calimyrna induced by gamma-(indole-3)-*n*-butyric acid (IBA), and (*e*) caprified Calimyrna.

2. Epidermal cells of Calimyrna induced parthenocarpic syconia at maturity were rectangular in radial section, with their long axes parallel to the surface of the syconium. Epidermal cells of Calimyrna caprified syconia, on the other hand, were rectangular in section, with their long axes perpendicular to the surface of the syconium. Epidermal cells in Kadota naturally parthenocarpic and caprified syconia were observed not to be different from each other but were like epidermal cells of Calimyrna induced parthenocarpic syconia.

3. The cuticle on the inner and outer epidermis of the receptacle, pedicel and perianth of the flowers, outer ovary wall, and integuments of both Calimyrna and Kadota caprified syconia was thicker than in Calimyrna and Kadota parthenocarpic syconia.

4. At each sampling, the thickness of the cortical parenchyma tissue and the number of cell layers contained in it were greater in most cases in both induced parthenocarpic Calimyrna and naturally parthenocarpic Kadota syconia than in syconia that had been caprified.

5. Six to seven layers of cells in the peripheral region of the cortex in both parthenocarpic and caprified Kadota syconia were identified as collenchyma. This characteristic is thought to be responsible for the superior canning qualities of the Kadota variety.

6. Although Kadota syconia contained a few more laticiferous ducts than Calimyrna syconia, no difference in size or development of these tissues existed between parthenocarpic and caprified figs.

7. Neither size nor number of the cells in the vascular bundles was markedly different in the various kinds of syconia examined.

8. The length and width of exocarp cells in the achenes of parthenocarpic syconia were considerably smaller than

corresponding cells in caprified syconia of both varieties.

9. The length and width of cells composing the outer mesocarp, as well as the thickness of this tissue, were smaller in induced parthenocarpic Calimyrna syconia than in caprified syconia, whereas the converse was true in the Kadota variety.

10. The development of the inner mesocarp, as measured by thickness, was considerably less in the parthenocarpic than in the caprified syconia of the respective varieties. Inner mesocarp cells of parthenocarpic and caprified Kadota and caprified Calimyrna syconia sclerified upon maturity, but no sclerification whatsoever took place in these cells in parthenocarpic Calimyrna syconia induced by IBA or PCPA. The content of calcium oxalate crystals was altered in the inner mesocarp cells in syconia induced with these growth substances.

11. The thickness and number of cell layers in the endocarp were somewhat greater in caprified than in parthenocarpic Kadota syconia. Although the endocarp was present in parthenocarpic Calimyrna syconia induced by PCPA, it remained parenchymatous in character and did not sclerify upon maturity of the syconium. At maturity, endocarp tissue was totally lacking in IBA-induced parthenocarpic Calimyrna syconia.

12. The integuments and nucellar tissue collapsed comparatively early, and there was no embryo or endosperm development in the achenes in parthenocarpic syconia.

13. The relationship between the splitting of maturing syconia and the development of the achenes is discussed. It is concluded that splitting is the result of growth of the fruitlets within the syconium, the degree of splitting being closely associated with certain environmental conditions.

14. The higher percentage of sugar in parthenocarpic than in caprified syconia is probably the result of the larger cortex and lack of seed development in the former.

15. A short discussion points out the specificity of the synthetic growth substances and the diverse reactions they induce. Although it is believed that the hormone which controls the growth of the syconium, in part, may be produced in the ovarian tissue, it is now known that endocarp tissue plays no part in this in IBA-induced parthenocarpic Calimyrna syconia, in which it is lacking.

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SOME ATTEMPTS TO ESTIMATE SPECIES DIVERSITY AND POPULATION DENSITY OF TREES IN AMAZONIAN FORESTS

G. A. BLACK, TH. DOBZHANSKY, AND C. PAVAN

Introduction

One of the striking differences between tropical forests and forests of temperate and cold lands is that the former contain, per unit area, considerably more species of trees than the latter. In particular, the diversity of species of trees in the Amazonian Hylea impressed naturalists, beginning with the early explorers and continuing down to our day. Quantitative data describing this diversity are, nevertheless, scarce in general and absent for the Amazonian region. DAVIS and RICHARDS (**2**) were apparently the first to undertake enumeration of species and of individual trees. They used five 122 × 122-meter (1.49 hectare) plots in the different "consociations" which they distinguished in the forests of British Guiana. Their results are impressive: 59 to 94 species among 460 to 919 trees 10 cm. or more in diameter at chest height per plot of the above size. RICHARDS (**8**) obtained figures of the same order of magnitude on three plots of similar size in the forests of Sarawak, Borneo; the difficulties which he encountered in delimitation of the species, however, were rather overpowering. In his monograph on the vegetation of Trinidad, BEARD (**1**) estimated the numbers of species (from 28 to 104) and the average numbers of individual trees (93 to 202) per acre in the different associations and faciations on the island. Although his estimates are based on far more ample information than was available to DAVIS and RICHARDS or to ourselves, the data are presented in a form that makes statistical analysis difficult. Estimates of the population densi-

ties of different species of trees have been given by VELOSO (9) for the forests of Teresopolis in the state of Rio de Janeiro.

In 1947 one of us (BLACK) made the first attempt to obtain numerical data on species diversity and population density of trees in a forest at Tefé, state of Amazonas. The stay of the junior authors at the Instituto Agronomico do Norte, Belém do Pará, in July and August, 1949, has been used to obtain further data of a similar kind for two types of forest in the state of Pará.

Material and methods

Two broad classes are distinguished among the many types of forests in the Amazonian Hylea. Terra firme forests are not reached by periodic inundations. Igapó or varzea forests grow on land which is waterlogged or inundated, owing either to annual floods which occur in the Amazon and its main tributaries or to the fresh-water tides which rise and fall twice each day in the delta of the Amazon and in Rio Pará.

We have made tree counts on 1-hectare (100 × 100 m.) plots marked off on reasonably uniform terrain in the forest by measured cord. From September 22 to 25, 1947, the first plot was studied in a terra firme forest near Tefé, Amazonas, on land which belongs to the Instituto Agronomico do Norte, and which, at the time this work was made, was in virgin condition. On July 18–20, 1949, the second plot was studied in an igapó some 200–300 m. from the Guamá River near Belém. This terrain, also belonging to the Instituto Agronomico do Norte, is within about 1 km. from the ruins of an old monastery abandoned some 80 years ago. The presence of cacao trees on this plot is a clear sign of human interference with the vegetation. The long abandonment of the locality, however, makes it probable that the climax vegetation is approximately restored. The third plot was studied on July 21–25, 1949, in the Mucambu terra firme forest of the Instituto Agronomico do Norte, near Belém. Despite its relative proximity to the city of Belém, this forest was apparently virgin, as attested by the presence of fairly numerous trees of species valued for their wood and much procured by woodcutters. The preservation of this forest is explained by its having been rather inaccessible until a recently constructed road was built in its vicinity; the terra firme of Mucambu is an island surrounded by igapó.

Our 1-hectare plots were subdivided by cords into ten 10 × 100-m. strips, except that on the second plot seven 10 × 100-m. strips and two larger ones were made. On each strip the trees were examined one by one, and every tree was marked by a machete as it was recorded. Trees less than 10 cm. in diameter at chest height ($31\frac{1}{2}$ cm. in circumference) were not recorded, except for the assaí and paxiuba palms. Assaí (*Euterpe oleracea*) is a caespitose form, with several trunks, usually less than 10 cm. in diameter individually, coming from the same root system. Each clump was counted as one individual. Paxiuba (*Iriartea exorrhiza*) has stilt roots, the trunk beginning often 2 m. or more above the ground. For trees with large plank buttresses it was also advisable to make measurements at above the usual height.

Recognition of species of trees in Amazonian forests is often difficult or impossible without examination of flowers and fruits, which are at any one time available only on a minority of individual trees. This difficulty exists even in the vicinity of Belém, where Dr. A. DUCKE and other botanists interested in forest trees have worked for many years. To

mitigate this difficulty, we, like DAVIS and RICHARDS (2) in their work in Guiana, resorted to the help of experienced "*mateiros*" or tree specialists. These men, although often illiterate, are able to identify most species of trees by their vernacular names which are in common use in a given region. The criteria which are used by *mateiros* for identification are chiefly the texture, smell, and taste of a bark sample freshly cut from the tree, presence or absence and quality of the latex and of bast fibers, and only secondarily the characteristics of the foliage, fruits, and flowers. Three *mateiros*, ANTONIO DA SILVA, FELICIANO ALVES FERREIRA, and ELIAS RAMOS DE ARAUJO, employees of the Instituto Agronomico, co-operated with us in studying the second and the third plots, in the vicinity of Belém. The first plot, at Tefé, was studied with the aid of MANOEL BRAGANÇA and ANTONIO DA SILVA. To these men we wish to express our appreciation of their conscientious collaboration.

The common names of trees used by *mateiros* usually, but not invariably, coincide with species recognized by botanists. For example, the name "muiraximbé" is applied to *Emmotum acuminatum* (Icacinaceae), and our collaborators used it, by mistake, also for *Dendrobangia boliviana* as well as *Minquartia guianensis* (Olacaceae), which is known as "acariquara." We have endeavored to test the accuracy of species recognition by asking the different men to name the same tree independently, without the others being within hearing distance. In most cases the names coincided; in the few cases, when they did not, we recorded all the names given. The discrepancies were in part due to the instability of the vernacular nomenclature, although a check against LE COINTE's manual (5) showed a large measure of consistency. We never corrected the names given to us by the *mateiros* on basis of literature sources. Furthermore, it was encouraging that our collaborators admitted their unfamiliarity with some rare species of trees and did not invent names for them.

The scientific equivalents of the common names of trees were often known but in some cases they were not, or the identification was in doubt on various grounds. We have endeavored to secure specimens of leaves and, where possible, of flowers and fruits of the individual trees in question. These specimens are preserved in the herbarium of the Instituto Agronomico do Norte, under numbers of the Black-1947 or Black-1949 series; some of these numbers are indicated in the following tables. The specimens were compared with determined herbarium sheets, and many specimens were shown to our colleagues, Drs. J. MURÇA PIRES, A. DUCKE, and R. L. FROES, whose help in determination has been invaluable. Some trees, especially from Tefé, nevertheless could not be determined as to species, as to genus, and a few even as to family.

In the following tables we list the best approximations to accurate botanical identifications of our material which we were able to secure. It must, however, be pointed out that for our purpose, which is estimation of the diversity of species of trees on known areas of the forest, correct differentiation of the species is vastly more important than their names. Such differentiation, we feel, has been accomplished in a satisfactory manner, with the notable exceptions of species of Myristicaceae, commonly known as "ucuuba" at Tefé, and of the species of *Protium*, known as "breu branco" and "breu vermelho" on the terra firme at Belém. The numbers given for these species are the best approximations which we could

make. The differentiation of species was, of course, assisted by the fact that we dealt in every case with sympatric species, i.e., growing in the same territory. This, as most taxonomists know, is a vastly easier task than delimitation of allopatric species.

Observations

Igapó forest

The counts made on our second plot, on igapó near Belém, are summarized in table 1. We recorded 564 trees, 430 of them with trunks between 10 and 20 cm. in diameter, and 134 with trunks more than 20 cm. in diameter. By far the most common species proved to be the assaí palm, of which 241 clumps were counted; this palm is common on igapó lands near Belém, as can be seen very clearly by observing the vegetation from a low-flying airplane. The next commonest species was the paxiuba palm (41), and the third a species of *Protium* (33). The total number of species recorded was 60. The numbers of species represented by 1, 2, 3, or other numbers of individuals on the plot are as follows:

Individuals	1	2	3	4	5	6	7	8	9	10	14	15	16	21	29	33	41	241
Species	22	9	7	2	2	2	4	2	1	1	1	1	1	1	1	1	1	1

More than a third (22) of all species found were represented by a single individual on our 1-hectare plot, and nine species were represented by two individuals each. Population densities of the rarer species are in some cases evidently less than one individual per hectare. Another plot of similar size in the same neighborhood would, accordingly, contain some species not encountered in our plot and would lack some species here recorded. This point will be considered in more detail in the discussion.

Terra firme forest at Belém

A summary of the counts made on the third plot, at the Mucambu terra firme forest near Belém, is given in table 2. The total number of trees counted was 423, of which there were 228 with trunks between 10 and 20 cm., and 195 with trunks more than 20 cm. in diameter. The number of species was 87, which appears to be higher than on the igapó (second) plot. Few species were encountered on both igapó and terra firme, although the distance between the two plots is hardly more than 2 km. Only one species, *Protium heptaphyllum* (breu branco), was common on both plots, and the identity of this species is not absolutely certain (see above). The palms which are common on igapó are rare on terra firme, assaí being absent on the latter. The numbers of species represented on this terra firme plot by various numbers of individuals are as follows:

Individuals	1	2	3	4	5	6	7	9	12	17	20	25	37	49
Species	33	15	15	3	4	2	3	4	1	2	1	1	2	1

No one species was very common; the highest frequency—49 individuals—which is only 11.6% of the total number of trees on the plot, belongs to a species of *Eschweilera* (matamatá branca). Two species of *Protium* were represented each by 37 trees; the uncertainty of classification of just these two species has been pointed out above. As many as thirty-three species, almost a third of the total, were represented by a single tree each.

TABLE 1

NUMBERS OF INDIVIDUALS OF TREES FOUND ON 1 HECTARE OF "IGAPÓ" NEAR BELÉM

FIRST OF TWO FIGURES INDICATES NUMBER WITH TRUNKS 10–20 CM. IN DIAMETER THE SECOND, THOSE ABOVE 20 CM. IN DIAMETER AT BREAST HEIGHT

Scientific name and herbarium reference (BLACK '49)	Common name	No. of individuals
Palmae		
Astrocaryum murumuru Wallace	Murumurú	6–4
Iriartea exorrhiza Mart.	Paxiúba	41–0
Euterpe oleracea Engel.	Assaí	241–0
Musaceae		
Ravenala guianensis (Endl.) Benth.	Sororoca	6–0
Moraceae		
Perebea sp. (8060)	?	0–1
Cecropia sp.	Imbauba vermelha	3–0
C. sp.	Imbauba branca	6–0
Pourouma sp.	Imbaubão, Torem	1–0
Ficus sp. (8059)	Caxinguba	0–1
Polygonaceae		
Coccoloba latifolia Lam. v. af.	Cau-assú	0–1
Anonaceae		
Guatteria sp.	Envira branca	1–0
Myristicaceae		
Virola surinamensis (Rol.) Warb.	Ucuúba	2–5
Hernandiaceae		
Hernandia sonora L.	?	2–0
Rosaceae		
? (8042)	?	1–0
Leguminosae		
Inga sp. (8049)	Ingá	1–0
I. edulis Mart.	Ingá cipó	11–4
I. sp. (8041)	Ingá branca	5–4
I. velutinosa Willd.	Ingá	1–0
Pithecolobium jupunum Harms.	Ingá de flor vermelha	6–1
P. cochleatum (Willd.) Mart.	?	2–6
Pentaclethra filamentosa Benth.	Pracaxí	6–10
Bauhinia splendens H.B.K.	Escada de jabotí	1–0
Pterocarpus rohrii Vahl.	Mututí	1–1
Vatairea parensis Ducke	Faveira	1–1
Macrolobium sp. (8038)	Araparí	0–1
Hymenaea parvifolia Huber.	Jutaí	0–2
H. sp. (8058)	Jutaí	1–0
Burseraceae		
Protium heptaphyllum (Aubl.) Marsh. (8052)	Breu branco nhambú	21–12
Meliaceae		
Carapa guianensis Aubl.	Andiroba	10–11
Guarea sp. (8040)	Farinha seca	1–2
G. sp.	Carne seca	2–3
Malpighiaceae		
Byrsonima sp.	Murucí	0–1
Dichapetalaceae		
Tapura sp.	?	1–0
Euphorbiaceae		
Hevea brasiliensis Muell. Arg.	Seringueira	0–4
Anacardiaceae		
Anacardium giganteum Engl. (8037)	Cajú-assú	0–2
Spondias mombin L.	Taperibá	0–5
Elaeocarpaceae		
Sloanea sp. (8039)	Envira de sapo	0–1
Tiliaceae		
Apeiba sp. (8051)	Pente de macaco	0–1
Bombacaceae		
Bombax munguba Mart.	Munguba	1–6
Ceiba pentandra (L.) Gaertn.	Sumauma	0–4
Quararibea guianensis Aubl.	?	1–1

TABLE 1—*Continued*

Scientific name and herbarium reference (BLACK '49)	Common name	No. of individuals
Sterculiaceae		
Theobroma cacao L.	Cacau	3–0
T. subincanum Mart.	Cupu-y	12–2
Dilleniaceae		
Davilla sp.	Cipó de fogo	2–0
Guttiferae		
Symphonia globulifera L. f.	Ananí	13–16
Rheedia sp.	Bacurí	2–1
Tovomita obovata Engl.	Manguerana	1–2
Lecythidaceae		
Eschweilera sp. (8061)	Matamatá	1–2
Gustavia sp.	Geniparana	2–5
Allantoma lineata (Berg) Miers	Churú	0–1
Combretaceae		
Buchenavia sp.	Mirindiba, Carará	1–1
Melastomataceae		
Bellucia sp.	Goiaba de morcego	1–0
Mouriria grandiflora DC.	?	1–0
Sapotaceae		
? (8044)	Guajará de leite	1–2
Ecclinusa sp. (8056)	Abiurana	0–1
Lucuma rivicoa Gaertn.	Cutite-tiribá	1–1
Boraginaceae		
Cordia sp. (8043)	Copeira	5–3
C. sp. (8036)	Envireira grão de galo	1–0
Fam. ?		
(8046)	Pacapuá	0–1
Fam. ?		
(8050)	Pupunharana	0–1
Total		430–134

The following tabulation shows how many species not previously encountered on the plot were discovered by examination of each of the ten 10 × 100-m. strips into which the 1-hectare plot was divided (see above):

Strip no.	I	II	III	IV	V	VI	VII	VIII	IX	X
"New" species	26	10	20	8	6	4	1	4	4	4

On the first strip 26 species were found, all of them obviously not seen before. On the second strip there were 10 "new" species not met with on the first strip (12 of the 26 species of the first were not encountered on the second). The third strip had an unexpectedly high number of "new" species, namely, 20. "New" species continued to appear, at a rate of about 4 species per strip, until the last strip was examined.

TERRA FIRME FOREST AT TEFÉ

Table 3 summarizes the data for the first plot studied, in terra firme forest near Tefé, Amazonas. The data obtained here are least satisfactory of the three plots studied, since only a minority of species have been botanically determined and since collection of herbarium specimens proved to be mostly impracticable. Furthermore, only trees with trunks more than 20 cm. in diameter

TABLE 2

NUMBERS OF INDIVIDUALS OF TREES FOUND ON 1 HECTARE OF "TERRA FIRME" FOREST AT MUCAMBÚ NEAR BELÉM. FIRST OF TWO FIGURES INDICATES NUMBER WITH TRUNKS 10–20 CM. IN DIAMETER; THE SECOND, THOSE ABOVE 20 CM. IN DIAMETER AT BREAST HEIGHT

Scientific name and herbarium reference (BLACK '49)	Common name	No. of individuals
Palmae		
Iriartea exorrhiza Mart.	Paxiúba	1–0
Oenocarpus distichus Mart.	Bacába	1–1
Moraceae		
Cecropia sp.	Imbauba de terra firme	2–1
Pourouma sp.	Imabubão	2–7
Perebea sp. (8136, Pires 1425)	Mão de gato	1–0
P. sp. (8123)	Mão de gato	0–3
P. *laurifolia* Trec. v. af. (8066)	Mão de gato	0–1
Olacaceae		
Minquartia guianensis Aubl.	Muiraximbé	0–1
Ptychopetalum olacoides Benth.	Muirapuama	6–0
Anonaceae		
Guatteria poeppigiana Mart.	Envira preta	1–0
Myristicaceae		
Virola melinonii (Benth.) Smith. v. af.	Ucuuba	2–1
Iryanthera sagotiana Warb.	Ucuuba	14–3
Lauraceae		
Aniba sp. (8161)	Louro rosa	0–2
Ocotea sp. (8127)	Louro cossueira	0–1
Genus ?	Louro amarelo de cheiro	0–1
Rosaceae		
Licania incana Aubl. (?)	Caripeirana	1–0
L. sp.	Macucú de sangue	0–2
L. macrophylla Benth.	Anoirá	0–1
Couepia sp. (8064)	Cumaté	2–1
Hirtella sp. (8159)	Cumaté	2–0
Leguminosae		
Vouacapoua americana Aubl.	Acapú	5–4
Tachigalia myrmecophila Ducke	Tachi preto	0–3
Bauhinia splendens H.B.K.	Escada de jabotí	2–0
Swartzia brachyrachis Harms v. af. (8089)	Mututí duro	2–1
Pithecolobium trapezifolium Benth.	?	1–0
P. sp. (8097)	Coração de preguiça	0–1
Inga alba Willd. v. af. (8103)	Ingá de rosca	1–0
I. lateriflora Miq. ? (8112)	Ingá	1–0
I. sp. (8153)	Ingá	0–1
Vatairea sp. ? (8119)	Angelim de pedra	0–1
Piptadenia sp. (8126)	Tambó, Timborana	4–3
Crudia sp. (8145, 8149)	Acupurana	1–1
Parkia pendula (Benth.)	Visgueiro	0–2
Humiriaceae		
Sacoglottis amazonica Mart.	Uxirana, Cumaté	1–2
Vantanea cupularis Huber	Uxirana, Achuá	0–2
Burseraceae		
Protium heptaphyllum (Aubl.) Marsh. (8116, 8062)	Breu branco	20–17
P. sp. (8101, 8105, 8062, 8117, 8135)	Breu vermelho	26–11
P. sp. (8163)	Breu vermelho	1–0
P. af. *giganteum* Engl. (8124)	Breu oriscca	3–3
P. neglectum Swart. (8158)	Breu sucurubá	1–3
Trattinickia burseraefolia Mart. (8072)	Breu	0–1
Meliaceae		
Carapa guianensis Aubl.	Andiroba	1–1
Guarea sp. (8069)	Carne seca	2–0
Trichilia sp. (8074)	Arú-arú	3–2
Vochysiaceae		
Vochysia guianensis Aubl.	Quaruba tinga	8–17
Qualea sp. (8104, 8125)	Pintadinho	1–2

TABLE 2—*Continued*

Scientific name and herbarium reference (BLACK '49)	Common name	No. of individuals
Dichapetalaceae		
Tapura singularis Ducke	Caferana	4–1
Euphorbiaceae		
Pogonophora schomburgkiana Miers	Arakapurí	1–1
Anacardiaceae		
Astronium sp. nov. (8085)	?	2–1
Anacardium giganteum Engl.	Cajú-açú	1–3
Thyrsodium paraense Huber	Pau de gafanhoto	0–1
Celastraceae		
Goupia glabra Aubl.	Cupiuba	1–16
Icacinaceae		
Dendrobangia boliviana Rusby	Muiraximbé	1–0
Emmotum acuminatum Miers	Muiraximbé	2–1
Elaeocarpaceae		
Sloanea sp. (8115)	Urucurana	0–1
S. guianensis Aubl.	Periquiteira	1–0
Tiliaceae		
Apeiba echinata Gaertn. v. af. (8139)	Pente de macaco	0–1
Sterculiaceae		
Theobroma subincanum Mart.	Cupú-y	8–1
Sterculia pruriens (Aubl.) Schum.	?	0–2
Caryocaraceae		
Caryocar glabrum Aubl.	Piquiarana	0–3
C. villosum Aubl.	Piquiá	0–1
Guttiferae		
Symphonia globulifera L. f.	Ananí	1–2
Tovomita obovata Engl.	Mangueranа	9–3
Lecythidaceae		
Eschweilera cf. *odora* (Poepp.) Miers	Matamatá branca	23–26
E. sp.	Matamatá preta	3–2
E. sp. (8162)	Matamatá sapeiro	0–1
E. cf. *krukovii* Smith	Matamatá jatereu	15–5
E. sp. (?)	Jarаninho	1–0
Gustavia sp.	Geniparana	1–0
Combretaceae		
Terminalia amazonica (Gmel.) Excell	Tanibouca	1–0
Myrtaceae		
Myrcia sp. (8151)	Cumaté	1–0
Melastomataceae		
Mouriria sagotiana Triana	Caa ingá	3–0
M. sp. (8080)	?	0–1
Miconia sp.	Buxuxú	2–0
Araliaceae		
Didymopanax morototoni (Aubl.) D. & P.	Morototó	0–1
Sapotaceae		
Manilkara huberi (Ducke) Standl.	Maçaranduba	1–4
M. sp. (8092)	Maparajuba	1–0
Micropholis acutangula (Ducke) Eyma	Abiurana	6–3
M. sp. (8133, 8146)	Gaujará, Mangaba de leite	1–2
Pouteria speciosa (Ducke) Bachni	Abíu	2–0
Lucuma sp. (8070, 8118)	Guajará, Mincira	2–1
L. sp. (8079)	?	0–1
Apocynaceae		
Aspidosperma desmanthum Benth.	Araracanga	3–1
Zschokkea aculeata Ducke	Pau de colher	1–0
Ambelania tenuiflora Muell.	Pepino	2–0
Verbenaceae		
Vitex triflora Vahl	Tarumá	1–0
Rubiaceae		
Chimarrhis turbinata DC.	Pau de remo	2–5
Total		228–195

were recorded. This, however, does not make the Tefé data noncomparable with those for the Belém plots, since the number of trees with trunks more than 20 cm. in diameter on the latter is known. The following tabulation indicates the numbers of species represented by various numbers of individuals with trunks more than 20 cm. in diameter on the three plots:

	Individuals	1	2	3	4	5	6	7	8	9	10	11	12	16	17	20	26	Total
Species	Plot I (Tefé)	42	14	9	3	..	..	2	1	2	1	2	1	..	1	1	..	79
	Plot II (Belém)	18	7	2	5	3	1	..	..	..	1	1	1	1	..	..	..	41
	Plot III (Belém)	31	11	10	2	2	..	1	..	..	..	1	..	1	2	..	1	62

The total number of species found at Tefé was 79. On a comparable basis, 41 species were found on the igapó and 62 species on the terra firme plot in Belém. A very limited number of the species found at Tefé are the same as those encountered on the Belém plots; the general composition of the samples is radically different. The predominant family on the Tefé plot is Myristicaceae, followed by Rosaceae and Moraceae, while at the Belém the commonest family on the terra firme plot is Burseraceae, followed by Lecythidaceae. A reservation, of course, must be made here—that the composition of the samples encountered on our plots does not necessarily characterize the whole terra firme floras of the Tefé and Belém regions, respectively.

The results obtained on the three plots indicate, if taken at face value, a greater diversity of species at Tefé than at Belém. It must be kept in mind, however, that while 230 trees were classified at Tefé, only 134 and 195 trees of the same diameter classes were classified on the two Belém plots. Now, in each case about half the species found were represented by single individuals, and, except on the igapó, less than a quarter were represented by more than three individuals each. Under such conditions, the number of species found in a sample depends to a great extent upon the number of individuals classified, and the observed differences in the diversity of species may well be spurious. This emphasizes the necessity of caution in drawing conclusions, on the basis of small samples, regarding the diversity of species in floras, faunas, or ecological associations.

Discussion

The number of species found in a random sample of a group of organisms is a function of several variables. Among these variables, the most evident ones are the total number of species of the group in question living in the territory in which, and at the time when, the sample is taken; the commonness and rarity of the species; and the size of the sample. A pioneer attempt to investigate the relationships between these variables was made by RAUNKAIER (**4, 7**). More recently the problem was taken up by FISHER *et al.* (**3**) and by PRESTON (**6**). The results obtained by the last-named author seem most satisfactory.

According to PRESTON, the commonness of species of a group of organisms follows a probability, or Gaussian, curve. That is, species with certain modal frequencies are likely to be the most numerous, while very common and very rare species are relatively few. PRESTON divides species into "octaves" of frequency. The first octave is composed of half the number of species represented in a

TABLE 3

NUMBERS OF INDIVIDUALS OF TREES FOUND ON 1 HECTARE OF "TERRA FIRME" FOREST OF EXPERIMENTAL PLOT OF ESTAÇÃO EXPERIMENTAL "ALTO SOLIMÕES," TEFÉ, AMAZONAS STATE, WITH DIAMETER GREATER THAN 20 CM. AT BREAST HEIGHT

Scientific name and herbarium reference (BLACK '47)	Common name	No. of individuals
Palmae		
Astrocaryum vulgare Mart. v. af.	Tucumã	1
Oenocarpus sp.	Bacaba	2
Moraceae		
Pourouma sp. (1520)	?	1
P. sp.	?	1
P. sp.	Torem	3
Pseudolmedia sp. ? (1514)	Mururé	3
Clarisia sp. (1394)	Guariuba	2
Brosimum pallescens Ducke v. af.	Amapá	3
Helicostylis asperifolia Ducke	?	2
? (1577)	Garrote	2
?	Muiratinga	3
?	Muiratinga	1
Olacaceae		
Minquartia guianensis Aubl.	Acariquara	3
? (1505)	?	2
Anonaceae		
Xylopia sp. (1508)	Envira surucucu	1
Guatteria sp. ?	Envira preta	2
?	Envira murteira	1
Myristicaceae		
Iryanthera sp.	Oití	12
I. sp.	Ucuuba vermelha	3
I. sp.	Ucuuba vermelha	7
I. juruensis Warb. (1589, 1393½)	Punã	11
I. sp.	Ucuuba vermelha	1
?	Ucuuba preta	8
?	Ucuuba	1
Virola sp. ?	Ucuuba branca	11
Lauraceae		
Aniba sp. (1512)	Louro rosa	3
? (1372)	Louro chumbo	1
Rosaceae		
Hirtella sp.	?	9
H. sp.	?	1
Licania incana Aubl. (1523)	Macucú	20
L. sp. (1517)	Caripérana	1
L. sp.	Caripé	1
L. sp.	Caripérana	2
?	Macucú	1
Leguminosae		
Inga sp.	Ingá	1
I. sp. (1503)	Ingá	1
Parkia sp.	Arara tucupí	1
Tachigalia sp.	Tachy	2
Ormosia sp. ?	Tento	1
O. sp. ?	Tenteiro	1
?	?	1
?	?	1
Burseraceae		
Protium sp.	Breu almescar	7
P. sp.	Breu branco	2

TABLE 3—*Continued*

Scientific name and herbarium reference (BLACK '47)	Common name	No. of individuals
Vochysiaceae		
Vochysia sp.	Quaruba	2
V. sp.	Quaruba	1
V. sp.	Pau branco	10
Erisma sp. ?	?	1
Euphorbiaceae		
Hieronyma laxiflora v. af.	?	9
Pogonophora schomburgkiana Miers	Aracapurí	4
Anacardiaceae		
Tapiria guianensis Aubl.	Itatapiririca	1
Celastraceae		
Goupia glabra Aubl.	Cupiuba	3
Elaeocarpaceae		
Sloanea sp.	Urucurana	1
S. sp. (1260)	?	4
Tiliaceae		
Lueheopsis rosea (Ducke) Burret (1515)	?	1
Bombacaceae		
Bombax sp.	Munguba	1
Caryocaraceae		
Caryocar villosum Aubl.	Piquiá	1
Caryocar glabrum Aubl.	Piquiarana	1
Quiinaceae		
Lacunaria jenmani (Radlk.) Ducke	Abiurana	2
Ternstroemiaceae (Theaceae)		
Laplacea semiserrata (Mart. & Zucc.) Cambess. (1506)	?	1
Lecythidaceae		
Eschweilera sp.	Matamatá	17
E. jarana (Hub.) Ducke (1485)	Jarana	1
Combretaceae		
Terminalia sp.	Tanibouca	2
Myrtaceae		
Myrcia sp. (1460)	Macucú cumaté	3
Melastomaceae		
Miconia sp.	?	2
Mouriria sagotiana Triana	Merauba	1
Apocynaceae		
Rauwolfia sp. (1519)		1
Himatanthus sp.	Sucuuba	1
Aspidosperma nitidum Benth. v. aff.	Carapanauba	2
Couma macrocarpa Barb. Rodr.	Sorva	1
Families unknown		
?		1
? (1521)		1
? (1348)	Amarelinho	4
?		1
?		1
?		1
?		1
?		1
?		1

sample by single individuals and by half the species represented by two individuals each. The second octave includes half the species represented by two, those represented by three, and half of those represented by four individuals in the sample. The third octave contains species represented by four to eight, the fourth octave by eight to sixteen, the fifth by sixteen to thirty-two individuals, etc. If the sample is large, one of the octaves will be represented by more species than the octaves to the right and to the left of it, which will contain progressively fewer species than the modal octave. But unless the sample is very large or the number of species very small, the curve will be truncated on the left, i.e., some of the species that are rare in the region or in the association studied will not be included in the sample. Some octaves of rare species will be "veiled." In very small samples even the modal octave may be veiled, and only less than half of the distribution will be exposed. The sample will consist mainly of the commonest species, and the largest number of species will be represented by single individuals.

The data obtained on the three plots studied by ourselves in Pará and in Amazonas, as well as on the five plots studied by DAVIS and RICHARDS (2) in British Guiana, are summarized in table 4 by "octaves." On all but a single plot, the octave of species represented by one or two individuals contains the largest number of species, and on that single plot this octave includes the same number of species as the octave next to the right. In accordance with the conception of PRESTON, the distribution curves constructed from our data and from those of DAVIS and RICHARDS are all truncated at the modal octave or even to the right of it. Only about a half, or less than half, of the species of trees which exist in a given forest type in the region studied have been met with and recorded on our plots. Most of the rarer species, and some of the moderately common ones, have been missed.

It may be noted that the plots of DAVIS and RICHARDS were larger (1.49 hectare) than ours (1 hectare). The numbers of individuals counted on their plots III, IV, and V were also larger than on other plots. This may be in part responsible for the large numbers of species recorded on these plots, as well as for the

TABLE 4

DISTRIBUTION OF SPECIES IN "OCTAVES" OF FREQUENCY. SAMPLES OF DAVIS AND RICHARDS ARE ALSO INCLUDED

Sample	<1	1–2	2–4	4–8	8–16	16–32	32–64	64–128	128–256	Total species	Total individuals
Tefé	>21	28	17½	4	6½	2	.....	.....	.....	79	230
Belém, Igapó	>11	15½	12½	10	5½	2½	2	.....	1	60	504
Belém, Terra firme	>16½	24	24	10½	5	4	3	.....	.....	87	423
D. & R., plot I	>11	16½	10	6	9½	3	2	1	.....	59	462
D. & R., plot II	>12½	17½	12½	13	7	4½	1	1	.....	68	400
D. & R., plot III	>14	23½	20½	13	9	3	5	1	.....	89	644
D. & R., plot IV	>15½	21	19½	17½	9	3	6½	2	.....	94	773
D. & R., plot V	>11½	15	11	10½	11	8	1	1	2	71	919

fact that the first three, or even four, octaves contain numbers of species which are not strikingly different from one another. Thus, the modal octaves of species may have been exposed on these plots; if so, DAVIS and RICHARDS have recorded somewhat more than half of all species which exist in the associations studied by them in these cases. The general conclusion is that the plots used by DAVIS and RICHARDS as well as by ourselves have been so small that the diversity of species of trees is underestimated by our data.

The data at our disposal suggest one further conclusion. The population density of a half or more of the tree species in Amazonian forests is likely to be less than one individual per hectare. This is a low population density by the standards of temperate zone forests. It is probable that at least some of the rare species of trees are cross-pollinated. Provided that the distribution of pollen by insects and by wind is about equally efficient in tropical and in temperate zone forests, the reproductive communities in tropical species of trees will contain on the average fewer individuals than reproductive communities in temperate zone species. If so, the genetically effective population sizes in many tropical species are likely to be limited. This would have a profound influence on their evolutionary patterns.

Summary

1. Counts of trees with trunks more than 10 (or 20) cm. in diameter have been made on three 1-hectare (100 × 100 m.) plots in the vicinity of Belém, state of Pará, and at Tefé, state of Amazonas. The species were delimited with the assistance of native tree specialists, and whenever possible identified by vernacular as well as by scientific names. On the three plots 60, 87, and 79 species have been recorded among 564, 423, and 230 individuals, respectively.

2. Analysis of the data with the aid of PRESTON's (**6**) "octave" method suggests that we have encountered probably no more than half the species which exist in the associations studied. Most of the rare, and some of the moderately common, species are missing on our plots. The population densities of many species of trees in the Amazonian Hylea are less than one individual per hectare.

It is our pleasant duty to acknowledge our indebtedness to the Director, Dr. FELISBERTO DE CAMARGO, and to the staff of the Instituto Agronomico do Norte for their hospitality and co-operation.

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EFFECTS OF PHOSPHORUS DEFICIENCY ON GROWTH AND METABOLISM OF SOYBEAN

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 613

SCOTT V. EATON

Introduction

This is the second paper of a series pertaining to the effects of phosphorus deficiency on the metabolism of plants. The first study (4) indicated that a shortage of phosphorus did not seriously affect the chemical constitution of sunflower plants. Carbohydrates accumulated in the minus-phosphorus stems, especially in the early stages, but this seemed to be due not to any disturbance in their metabolism but to a greater leaf-stem ratio in comparison with the plants on complete-nutrient solution. None of the nitrogen fractions was high in phosphorus-starved stems. The plants seemed to grow as much as the shortage of phosphorus allowed without any serious disturbance of their metabolism.

The above-mentioned data are in contrast to the results of other investigators (5, 7, 8, 15) who have reported serious effects of phosphorus deficiency on plant metabolism. The main effects found were the accumulation in phosphorus-starved plants of carbohydrates and the soluble forms of nitrogen: nitrate, ammonia, amino acids, and amides. On the other hand, RICHARDS and TEMPLEMAN (15) found large amounts of amides and amino acids in low-phosphorus barley leaves when nitrate was still quite low.

It will be shown below that the data of the present investigation contrast in several respects with those obtained in the first study of this series. They are more in accord with the results of the other investigators referred to above, although there are certain points of contrast.

Material and methods

Soybean plants of the Manchu III variety were grown in pure quartz sand with the same nutrient solution and by essentially the same methods as previously described (4). The only modification was that, in order to prevent blooming before the plants had reached the desired stage of phosphorus deficiency, the photoperiod was increased to about 18 hours with 200-watt incandescent Mazda bulbs placed about 2 feet above the plants. The seed was obtained from the University of Wisconsin through the kindness of Professor L. F. GRABER.

The seed was planted on April 10, 1947, and the plants were harvested on May 15, May 26, and June 4. The number of plus- and minus-phosphorus plants of the first harvest were 91 and 210, respectively; of the second, 84 and 147; and of the third, 70 and 104.

Essentially the same methods as in the previous work (4) were used in sampling, extraction, and chemical analysis. Alkalinity was obtained with sodium hydroxide in the determination of ammonia, amides, and nitrates, and no potassium carbonate was used. This is the procedure followed by BURKHART (1) in determining ammonia and amides. It should be stated that the reference in the previous paper to COTTON'S (3) method of determination of phosphorus is incorrectly cited.

Results

Appearance and size of plants

About 2½ weeks after planting the seed the leaves of many minus-phosphorus plants were greener than the plus-phosphorus ones. Also, some of the cotyledons of the former plants were chlorotic. At this time the size of plants in the two groups was about the same. Later the lower leaves of the phosphorus-starved plants became chlorotic. The pattern of chlorosis was a gradual decrease in chlorophyll throughout the leaf in the interveinal areas. Later the veins also became chlorotic, and the leaf soon died. Chlorosis was also a feature of the lower leaves of the plants on complete nutrient, but it occurred later and was not so prevalent. It seems that the basal leaves of soybean plants tend to lose their chlorophyll even under satisfactory conditions for growth.

The minus-phosphorus plants were smaller than the plus-phosphorus ones, with smaller leaves and shorter, thinner, and harder stems. At later stages of the deficiency the size differences were very great (table 1, and figs. 1, 2).

TABLE 1

FRESH WEIGHT OF STEMS (GRAMS PER 100 PLANTS), PERCENTAGE WATER, PERCENTAGE DRY WEIGHT

Stem level*	Fresh weight		Percentage water		Percentage dry weight	
	Minus P	Plus P	Minus P	Plus P	Minus P	Plus P
	First harvest					
Upper	54	120	84.5	89.5	15.5	10.5
Middle	61	110	81.5	86.6	18.5	13.4
Lower	97	147	79.6	84.2	20.4	15.8
	Second harvest					
Upper	67	279	80.3	87.5	19.7	12.5
Middle	79	252	77.2	83.8	22.8	16.2
Lower	133	255	77.4	82.1	22.6	17.9
	Third harvest					
Upper	78	496	80.1	86.9	19.9	13.1
Middle	92	436	75.8	83.1	24.2	16.9
Lower	160	395	76.9	80.7	23.1	19.3

* Total stem length cut into three segments of equal length.

Weight of stems, percentage water, percentage dry weight

Table 1 gives the fresh weight of the stems and their percentage water and dry weight. Weights were also taken of leaves and roots; but, since the differences in these parts were similar to those of the stems, the data are not given. The stems

of the minus-phosphorus plants weighed less than the plus-phosphorus ones. The differences were very great at the second and third harvests. There was a gradient in weight of the phosphorus-deficient stems, increasing from the top to the bottom level. On the other hand, after the first harvest the complete-nutrient stems weighed more in their upper third, and at the third harvest there was a decreasing weight gradient from top to bottom.

The minus-phosphorus stems were lower in moisture (higher in percentage dry weight) than the plus-phosphorus

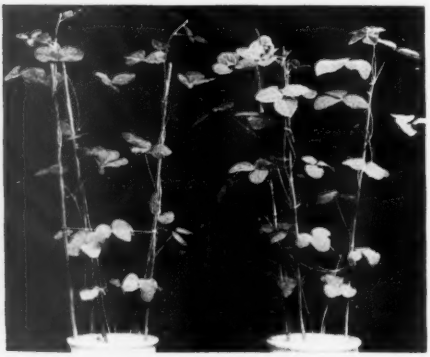

FIG. 1.—Soybean plants at first harvest, 35 days from planting of seed: minus-phosphorus on left, plus-phosphorus on right.

ones at all three levels. The percentage water content of the latter stems decreased regularly from top to bottom. This was not true of the former except at the first harvest, although at each harvest there was more water at the upper level than at the middle and lower ones.

Phosphorus deficiency affected the tops more than the roots, so that the top-root ratios of the phosphorus-starved plants were lower than for those on complete nutrient. These ratios for the first, second, and third harvests were 2.694, 2.460, and 2.694, respectively, for the minus-phosphorus plants and 3.964, 6.273, and 5.881 for the plus-phosphorus ones. The differences in favor of the plants grown in a complete-nutrient solution were somewhat exaggerated, especially at the later harvests, because of the death of more of the lower leaves of the minus-phosphorus plants than of plus-phosphorus ones. Even at the first harvest, however, before any leaves had died, the ratio for the latter plants was decidedly larger.

TOTAL SOLIDS, NITROGEN FRACTIONS

The chemical constituents of the stems were figured on both dry- and wet-weight bases. To save space, however, only the former data are given (tables 2–5). Since the percentage dry weights of the minus-phosphorus stems were greater than those of the plus-phosphorus ones, the chemical data for the former are lower relative to the latter than when figured on the fresh-weight basis.

There were no consistent differences in the soluble solids of the two sets of stems (table 2), but, figured on the fresh-weight basis, they were considerably higher in the phosphorus-starved stems. In both groups the soluble solids were higher at the upper level, and, except in the minus-phosphorus stems of the third harvest, there was a decreasing concentration from top to bottom. In general, the percentages of total nitrogen and 70%-alcohol-soluble nitrogen were larger for the phosphorus-starved than for the complete-nutrient stems. The differences in the two sets in soluble nitrogen were considerably larger in the second and third harvests than in the first. Much more of the total nitrogen was present in the soluble form in the minus-phosphorus than in the plus-phosphorus stems; on a whole-stem basis more than two-thirds of the total nitrogen was present in this form at each harvest in the former

stems and less than half in the latter. There were no consistent differences in the two sets of stems in 70%-alcohol-insoluble nitrogen, but on a fresh-weight basis there was in general a higher concentration of this fraction in the phosphorus-deficient stems.

Ammonia and amides were much higher in the minus-phosphorus than in the plus-phosphorus stems (table 3). Only a trace of ammonia was found at the upper level of the latter stems and none at all at the middle and lower levels. Nitrate percentages were much larger in the complete-nutrient stems except at the lower level. These fractions were determined on the stems of the third harvest only.

It should be stated that ammonia, amides, and nitrates were determined about 19 months after harvest. This is a rather long interval. WEBSTER (**17, 18**) noted changes in the nitrogen compounds of stored alcoholic extracts. The main change found was a decrease in amino nitrogen and an increase in ammonia; nitrates did not change. It is possible that the ammonia percentages of table 3 are too high. Total soluble nitrogen which was determined promptly, however, was, on the average, much higher in the minus-phosphorus stems of each harvest. Since this composite fraction included the fractions of table 3 and also amino acids and other soluble nitrogen compounds, it is probable that the data of this table represent essentially the differences in the two sets of stems.

The gradients of the nitrogen fractions of tables 2 and 3 were quite different in the two sets of stems. In general, these fractions of the plus-phosphorus stems decreased from top to bottom. On the other hand, in the minus-phosphorus stems they were generally higher at the middle or lower level.

The percentages of total sugars, reducing sugars, and sucrose were larger in the phosphorus-deficient than in the complete-nutrient stems at the first harvest (table 4). The differences were less at later harvests and at the third harvest were, in general, in favor of the latter stems. Calculated for whole stems, the

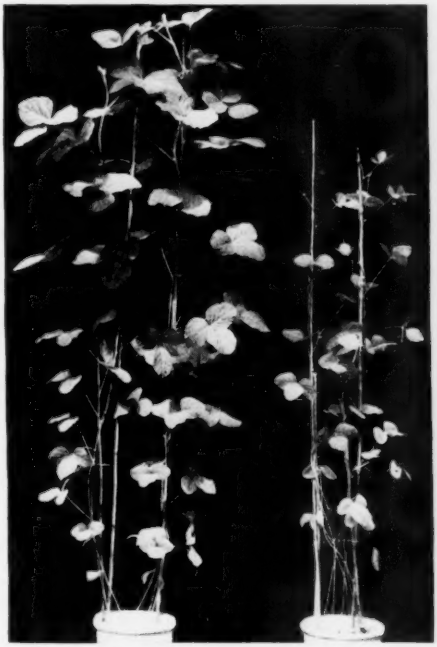

FIG. 2.—Soybean plants at second harvest, 45 days from planting of seed: minus-phosphorus on right, plus-phorphorus on left.

percentages of total sugars in the minus- and plus-phosphorus stems were, for the first harvest, 2.309 and 0.980, respectively; for the second, 1.936 and 1.543; and for the third, 1.326 and 1.858. Starch was uniformly higher in the phosphorus-starved than in the complete-nutrient stems at each harvest. The amount of starch in the former stems and the differences in the two sets increased

at later harvests. The increase of starch in the minus-phosphorus stems was greater than could be accounted for by the decrease in sugars. Including starch, these stems were at all times much higher in total carbohydrates than the plus-phosphorus ones.

Total sugars and reducing sugars were uniformly higher at the upper level of each set of stems, although in most cases there was not a regular decrease from top to bottom. The sucrose gradients were variable, and in some cases none was exhibited. The concentration of starch in the complete-nutrient stems decreased regularly from top to bottom at each harvest. On the other hand, in the phosphorus-starved stems it increased in the

TABLE 2

SOLUBLE SOLIDS, TOTAL N, SOLUBLE N, INSOLUBLE N (PERCENTAGES ON DRY-WEIGHT BASIS)

STEM LEVEL	70%-ALCOHOL-SOLUBLE SOLIDS		TOTAL N		70%-ALCOHOL-SOLUBLE N		70%-ALCOHOL-INSOLUBLE N	
	Minus P	Plus P	Minus P	Plus P	Minus P	Plus P	Minus P	Plus P
	First harvest							
Upper	30.0	33.6	2.21	2.70	1.00	1.11	1.21	1.59
Middle	22.1	23.0	3.01	2.11	2.21	1.16	0.80	0.95
Lower	19.5	17.4	2.63	1.43	1.92	0.70	0.71	0.73
	Second harvest							
Upper	24.8	29.4	1.88	2.14	1.15	0.87	0.73	1.27
Middle	19.2	18.3	3.23	1.53	2.16	.68	1.07	0.85
Lower	18.6	15.1	2.96	1.27	1.92	0.52	1.04	0.75
	Third harvest							
Upper	24.6	29.9	2.28	2.34	1.41	1.16	0.87	1.18
Middle	19.3	19.2	3.12	1.73	2.08	0.91	1.04	0.82
Lower	19.5	15.4	3.20	1.16	2.15	0.34	1.05	0.82

TABLE 3

AMMONIA, AMIDES, NITRATES (PERCENTAGES ON DRY-WEIGHT BASIS, THIRD HARVEST)

STEM LEVEL	AMMONIA N		AMIDE N		NITRATE N	
	Minus P	Plus P	Minus P	Plus P	Minus P	Plus P
Upper	0.030	0.005	0.372	0.298	0.176	0.363
Middle	.028	.000	.516	.179	.182	.434
Lower	0.051	0.000	0.496	0.059	0.262	0.172

same direction at the first and second harvests. No definite gradient was exhibited at the third harvest, but there was more starch at the middle and lower levels than at the upper.

All the phosphorus fractions were higher in the plus-phosphorus than in the minus-phosphorus stems of all harvests (table 5). In each set the concentrations were greater at the upper level. In the complete-nutrient stems all the phosphorus fractions decreased regularly from top to bottom at each harvest, but in certain cases in the phosphorus-starved stem there was a slightly higher concentration at the lower than at the middle level. More of the total phosphorus was found in the soluble form in the minus-phosphorus than in the plus-phosphorus stems. The values of this fraction, calculated as percentage of total phosphorus for whole stems in the phosphorus-starved and complete-nutrient sets were, for the first harvest, 45.34 and 31.64, respectively; for the second, 47.69 and 35.78; and for the third, 44.95 and 33.07.

TABLE 4

CARBOHYDRATE FRACTIONS (PERCENTAGE ON DRY-WEIGHT BASIS)

Stem level	Total sugars		Reducing sugars		Sucrose		Starch	
	Minus P	Plus P	Minus P	Plus P	Minus P	Plus P	Minus P	Plus P
	First harvest							
Upper	4.41	2.09	3.52	1.84	0.89	0.25	2.25	1.44
Middle	1.34	0.65	0.43	0.32	0.91	.33	3.34	0.47
Lower	1.59	0.47	0.48	0.24	1.11	0.23	5.52	0.41
	Second harvest							
Upper	2.65	3.36	1.36	2.51	1.29	0.85	4.64	0.94
Middle	1.63	0.93	0.37	0.32	1.26	.61	7.29	0.59
Lower	1.69	0.83	0.53	0.34	1.16	0.49	8.19	0.52
	Third harvest							
Upper	2.13	3.34	1.11	2.54	1.02	0.80	6.11	1.19
Middle	1.01	1.28	0.29	0.42	0.72	.86	9.27	0.95
Lower	1.14	1.36	0.24	0.38	0.90	0.98	7.96	0.90

Discussion

In a previous paper (**4**) a rather detailed discussion was given of the effects of phosphorus deficiency on the sunflower. Since the effects on the soybean and sunflower were similar in many respects, several points included in the sunflower report will be omitted from the present discussion. These are phosphorus-deficiency symptoms, top-root ratios of plus- and minus-phosphorus plants, and the effects of phosphorus deficiency on the phosphorus fractions. The publication on sunflower also contains a more complete bibliography than is given in

the present paper. In a future paper it is planned to report the differences among plants in effects of phosphorus deficiency, especially the three plants of this project—sunflower, soybean, and black mustard.

EFFECTS OF PHOSPHORUS DEFICIENCY ON CHEMICAL CONSTITUTION

CARBOHYDRATE FRACTIONS.—The minus-phosphorus stems were at all harvests high-carbohydrate stems in comparison with the plus-phosphorus ones. Reducing sugars and sucrose were much higher in the former than in the latter at the first harvest. The differences decreased at later harvests and at the third harvest in general favored the complete-nutrient stems. But the percentages of starch in the phosphorus-starved stems were much larger at each harvest, and their starch content increased with age. Calculated for whole stems the percentages of total carbohydrate of minus- and plus-phosphorus stems were, for the first harvest, 6.16 and 1.70, respectively; for the second, 8.80 and 2.20; and for the third, 9.33 and 2.85.

There are two main explanations in the literature for the accumulation of carbohydrates as a result of phosphorus deficiency. ECKERSON (5) explained the high carbohydrate content of phosphorus-starved tomato plants by the interference with protein synthesis at the nitrate-reduction stage. She reported that reducase was entirely absent from the tops of minus-phosphorus plants 2 weeks after the start of the no-phosphorus treatment. Both carbohydrates and nitrates accumulated, and the plants assumed the characteristics of nitrogen-

TABLE 5

PHOSPHORUS FRACTIONS (PERCENTAGE ON DRY-WEIGHT BASIS)

STEM LEVEL	TOTAL P		70% ALCOHOL-SOLUBLE P		70% ALCOHOL-INSOLUBLE P	
	Minus P	Plus P	Minus P	Plus P	Minus P	Plus P
	First harvest					
Upper	0.107	0.399	0.046	0.115	0.061	0.284
Middle	.042	.241	.018	.083	.024	.158
Lower	0.030	0.172	0.016	0.057	0.014	0.115
	Second harvest					
Upper	0.061	0.274	0.027	0.091	0.034	0.183
Middle	.028	.174	.016	.065	.012	.109
Lower	0.033	0.146	0.015	0.055	0.018	0.091
	Third harvest					
Upper	0.048	0.270	0.022	0.088	0.026	0.182
Middle	.032	.183	.014	.059	.018	.124
Lower	0.036	0.149	0.016	0.051	0.020	0.098

starved ones. The early phase of phosphorus deficiency was regarded as resulting from this loss of the power to reduce nitrates.

On the other hand, RICHARDS and TEMPLEMAN (15) found that the most marked characteristic of phosphorus starvation in barley leaves was the accumulation of amino acids and amides, especially amides. This occurred while nitrates were about normal in amount and seemed to be caused by the interference with protein synthesis at the amide stage owing to the deficiency of phosphorus. Protein content was low even at early stages of growth. The similarity of many of the symptoms of nitrogen and phosphorus deficiencies was regarded as owing to the inability to synthesize adequate amounts of protein rather than to failure of nitrate reduction. At the same time that amino acids and amides accumulated, there was a large increase in reducing sugars, according to parallel work of GREGORY and BAPTISTE. It was suggested that amides and sugars are the simple building materials of proteins. With protein synthesis interrupted at the amide stage, both amides and sugars accumulated.

The explanation of RICHARDS and TEMPLEMAN, rather than that of ECKERSON, seems to account for the accumulation of carbohydrates in the minus-phosphorus soybean stems of the present experiment. Nitrates did not accumulate in the phosphorus-starved stems of the third harvest except at the lower level. Calculated for whole stems the percentage of nitrates in the minus-phosphorus stems was 0.23 and in the complete-nutrient stems, 0.42. Phosphorus deficiency did not seem to interfere with nitrate reduction so that protein synthesis could proceed to the amino acid stage. But since phosphorus is a component of nucleoproteins of the nucleus and of lecithoproteins of the cytoplasm, these proteins could not be synthesized. Amino acids presumably accumulated and in part changed to amides, which also accumulated (table 3). Since, according to much investigation (2, 14) carbohydrates are needed for synthesis of proteins from amides, it would be expected that carbohydrates would be high in the minus-phosphorus stems. It seems that protein synthesis is often channeled through amides. This has been studied to a greater extent in the resynthesis of proteins from the hydrolytic products of proteins of leaves or seeds. Part of the amino acids resulting from the hydrolysis of storage proteins of germinating seeds may be resynthesized directly into proteins in the seedling; but part is changed to amides, and these are used in protein synthesis. Even in the primary synthesis of proteins with nitrates as the nitrogen source, amides may be important. STEWARD and STREET (16) regarded protein synthesis in potato disks as canalized through glutamine. Although it was regarded that amino acids or asparagine might serve as a source of ammonia for glutamine synthesis, reduction of nitrates was thought to be a possible source.

NITROGEN FRACTIONS.—Total soluble nitrogen and two of its components, ammonia and amides, were much higher in minus-phosphorus than in plus-phosphorus stems. Amino acids were not determined, but they also probably accumulated in these stems, since concentrations of amino acids and amides in plant tissues usually run more or less parallel.

ECKERSON (5) gave two possible explanations of amino acid and amide accumulation in phosphorus-starved plants. They may have been formed early when there was still some reducase activity but when certain conditions necessary for

their synthesis into proteins were lacking; or they may have resulted from proteolysis late in phosphorus starvation. As discussed above, RICHARDS and TEMPLEMAN (15) explained the accumulation of these compounds in phosphorus-deficient barley leaves by the interruption of protein synthesis at the amide stage owing to the phosphorus shortage. This corresponds essentially to the first explanation of ECKERSON, phosphorus being the lacking material necessary for protein synthesis.

A study of the data of the present experiment indicates that both of ECKERSON's explanations are needed to explain adequately the differences in nitrogenous fractions in the two sets of stems. With phosphorus deficient there would be an interruption of the synthesis of nucleo- and cytoplasmic-proteins at the amide stage, so that it would be expected that amino acids and amides would accumulate in the phosphorus-starved stems. This has been discussed above. A consideration of the gradient differences of the two groups of stems, however, indicates that ECKERSON's second explanation, proteolysis, is also needed for an adequate interpretation. The amide gradient was positive downward (concentration decreased from top to bottom) in the plus-phosphorus stems, but in the minus-phosphorus ones the concentration was largest at the middle level, although at the lower level it was much greater than at the upper (table 3). The relative numbers of living cells were probably greater at the upper level in each set of plants, so that the synthesis of organic nitrogen compounds should have been more rapid in this level. Amides may be regarded as a step on the way to proteins. Since synthesis beyond the amide stage was retarded in the phosphorus-starved plants by a deficiency of this element, the gradient of this fraction should have been in the same direction in these stems as in the complete-nutrient ones but even steeper. Instead, amides were more concentrated at the middle level. Perhaps proteolysis was taking place, the resulting compounds being translocated to the tops of the plants and used in growth. Although the minus-phosphorus plants were decidedly shorter than the plus-phosphorus ones, still they elongated remarkably. At the third harvest the height of the tallest plant of the former set was 50 inches, of the latter, 69. On the basis of the above reasoning, the amide gradient—and also the soluble nitrogen gradient, which would include, in addition to amides, amino acids and other soluble nitrogenous compounds (table 2)—may indicate upward transport in the phosphorus-starved stems but downward transport in the complete nutrient.

Although the above interpretation of the gradients may be correct, much more work would be necessary to establish it. In particular, ringing experiments should be performed. MASON and MASKELL (12, 13) showed definitely that sugars were translocated downward in the bark of the cotton stem along a marked positive gradient. When a ring was made below the leaves, sugars accumulated in the stem above the ring and even in the leaves 2 feet distant. Preliminary work of MASKELL and MASON (9) indicated that nitrogenous compounds were transported downward in the cotton stem on a similar basis. But later work (10) showed that the gradients of total nitrogen and organic crystalloid nitrogen were negative downward (increasing concentration from top to bottom). It was suggested that the gradient of the translocatory forms of nitrogen was positive downward, but that it was masked by a greater negative gradient of storage nitrogen. Further work (11) tended to confirm this suggestion.

On the other hand, ENGARD (**6**) regarded the positive downward gradients of nitrogenous fractions of raspberry canes growing in soil as due to the greater number of living cells in the upper parts. Nitrates were translocated freely in the raspberry canes and were available in all living cells for the synthesis of organic nitrogenous compounds. Since the gradient of living cells was positive downward, that of the nitrogenous compounds was in the same direction. That the gradients did not indicate downward transport was indicated by lack of accumulation above a ring and the failure of a ring to reverse the gradient or even make it level.

In the absence of ringing experiments, therefore, no positive statement can be made as to the meaning of the nitrogen gradients in the soybean stems. Since the gradient of living cells was probably in the same direction in the two sets of stems, however, it seems significant that the gradients of nitrogen fractions are in reverse directions.

Summary

1. Sand cultures were used in a study of the effects of phosphorus deficiency on soybean plants. At an early stage of development the main symptom was the deeper green color of the leaves. Later a general chlorosis developed in the interveinal areas of the lower leaves. Finally, the veins became chlorotic, and the leaf soon died. The deficient plants were small, with small leaves and thin, hard stems. The tops were affected more than the roots, so that the top-root ratio of the minus-phosphorus plants was lower than that of plus-phosphorus ones.

2. Phosphorus-deficient stems were low in moisture. The stems of the younger plants were high in total sugars, reducing sugars, and sucrose. These differences in the two sets of stems decreased in the older plants, and at the third harvest all these fractions were in general higher in the complete-nutrient ones. There was much more starch in the minus-phosphorus than in the plus-phosphorus stems at each harvest, and the percentage of starch in the former stems and the differences in the two sets increased with age. Including starch, the phosphorus-starved stems were at each harvest high-carbohydrate ones. The accumulation of carbohydrates in these stems is interpreted as owing to the interference with protein synthesis at the amide rather than at the nitrate-reduction stage as has been found in other plants. Amides accumulated in the minus-phosphorus stems, but nitrates were higher in the plus-phosphorus ones.

3. Total nitrogen, soluble nitrogen, ammonia, and amides were higher in the phosphorus-starved stems; percentages of nitrates were larger in the complete-nutrient ones. The gradients of these fractions were, in general, positive downward in the latter stems, but in the former the concentrations were higher at the middle or lower level. More of the total nitrogen was present in the soluble form in the minus-phosphorus than in the plus-phosphorus stems. The accumulation of total soluble nitrogen and amides in the former stems is thought to be caused both by the interruption of protein synthesis at the amide stage and by proteolysis.

4. There were much larger percentages of total phosphorus, soluble phosphorus, and insoluble phosphorus in the complete-nutrient stems than in the phosphorus-deficient ones. The soluble phosphorus of the latter stems made up a larger percentage of the total phosphorus than in the former ones.

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GROWTH-PROMOTING AND GROWTH-RETARDING SUBSTANCES IN POLLEN FROM DIPLOID AND TRIPLOID APPLE VARIETIES[1]

POUL LARSEN AND SIN MIN TUNG

Introduction

Triploid apple varieties are generally considered much inferior to diploids as pollinators (3). Among the causes of this inferiority may be mentioned the lower production of pollen grains per flower, the lower germination percentage of the pollen grains formed, and the incapacity of the sperm nuclei to accomplish normal fertilization. The lower pollinating value of the pollen of triploids might be associated with differences in its content of specific substances as compared with

[1] The major part of the present work was carried out in the Laboratory of Plant Physiology, University of Copenhagen, and in the Department of Horticulture, Royal Danish Veterinary and Agricultural College, Copenhagen, Denmark. Additional experiments at the University of Chicago were supported by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

pollen from diploids. Such substances might influence either the germination of the pollen grains or the course of certain processes taking place in the style or ovary after pollination.

The presence of auxin in pollen or anthers has been demonstrated by numerous workers (**6, 9, 11, 12, 13, 18, 21, 25, 27, 30, 31**). Two different processes have been assumed to be regulated by the auxin contained in the pollen grain: the elongation of the pollen tube and the enlargement of the fruit. In some plants, as orchids, the pollen grains seem to contain enough auxin to secure normal germination, because germination is not improved by addition of synthetic auxins to the medium (**4**). In other cases the auxin content must be considered a limiting factor; for example, germination and tube growth in *Antirrhinum* and *Bryophyllum* can be improved by addition of synthetic auxins (**24**).

Substances other than auxins which may influence germination and tube growth have also been found in pollen grains but will not be discussed here.

GUSTAFSON (**7**) was of the opinion that the growth of ovules after fertilization is induced by auxin contained in the pollen grains and transferred to the ovules through the pollen tubes. Once growth of the embryos had started, they were assumed to produce enough auxin to account for further enlargement of the fruit. VAN OVERBEEK *et al.* (**28**) and MUIR (**21**) found it unlikely that the amount of auxin which could be carried to the ovules from the average number of pollen grains in an ordinary pollination would be sufficient to account for the increase in auxin content of the pistil after fertilization. They assumed that the pollen supplies the pistil with an agent which stimulates the formation of auxin from a precursor already present in the pistil. For further discussion of these questions see (**8**).

In connection with a study of the pollination of some Danish apple varieties, we considered it desirable to ascertain whether any correlation existed between the pollinating value of the pollen and its content of growth-promoting and growth-retarding substances.

Material and methods

Four varieties of apple were used, the diploid ($n = 34$) James Grieve, and the triploids ($n = 51$), Graastener, Tompkins King, and Belle de Boskoop.

Since extractions and germination experiments could not be completed within the flowering season, it was necessary to collect a suitable amount of pollen and store it for later use. Several hundred flowers of each variety were kept in open glass dishes placed in the laboratory near a window facing east. They were exposed to sunlight for about 3 hours every day. After 4 days the flowers had dried; the pollen was collected and stored in a desiccator at about 6° C.

Pollen germination was studied in hanging drops of a liquid substrate rather than in agar media. Agar has in some cases (**10**) proved to be inferior to a liquid solution as a medium for pollen germination. The medium used for germination experiments contained per liter:

	Mg.
$Ca(NO_3)_2$	35
KCl	8
$MgSO_4 \cdot 7H_2O$	8
H_3BO_3	7 (**23**)
KH_2PO_4	3.5
Ferric tartrate	0.8
Malic acid	0.7 (**22**)
$MnCl_2 \cdot 4H_2O$	0.07
$CuSO_4 \cdot 5H_2O$	0.008
$ZnSO_4 \cdot 7H_2O$	0.008

To this basic medium was added 21.5 or 30% sucrose. The pH value of the

solution was adjusted to 4.9 or 6.9 by the addition of KOH or HCl. Germination percentages were determined on the basis of 200–500 grains counted after 48 hours at 20° C.

For extraction of growth substances 5 ml. of peroxide-free ether were added to 100 mg. of dried pollen in a test tube which was kept at room temperature. The ether was changed after $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, 19, 24, 163, and 168 hours (7 days), 5 ml. of fresh ether being added each time. The first two batches of extract were combined and denoted "1st hour." Batches Nos. 3 and 4 were likewise combined and denoted "2d hour." The combined batches Nos. 5 and 6 were denoted "3d to 24th hour," and the last two batches were labeled "25th to 168th hour." Each extract, consisting of 10 ml., was fractionated into an acid and a neutral component as described by LARSEN (**17**), sodium bicarbonate and tartaric acid being used for adjustment of pH values. Thus, from each sample of 100 mg. of pollen, four neutral fractions (15 ml. each) and four acid fractions (25 ml. each) were obtained.

Anther extracts were made in a similar way. One thousand anthers, weighing 1.36–1.86 gm., were extracted with 10 ml. of ether which was changed after about 3 hours and again after 24 hours. The two batches of ether were combined.

Extracts were transferred to agar by BOYSEN JENSEN's dropping method (**1, 2**) for growth-substance assay and tested on soil-grown *Avena* plants (**15**). Only curvatures below the maximum angle were used for calculation of the auxin activity.

Growth-promoting activity of acid fractions of pollen extracts are expressed as μgm. of indoleacetic acid. This is done by means of an "activity curve" obtained by plotting curvatures against concentrations of indoleacetic acid (fig. 1 in **17**).

After the growth-substance determinations had been completed at the University of Copenhagen, it was found by the senior writer at the University of Chicago that tartaric acid (used to acidify the aqueous solutions before shaking with ether) seemed to contain a growth inhibitor. This became evident in experiments of the following type: Pure ether was subjected to the same treatment as the pollen extracts, whereby a neutral and an acid ether fraction were obtained. Various amounts of these fractions were dropped on agar together with a known amount of indoleacetic acid. The auxin activity of the agar was determined in the *Avena* test. It was found that addition of ether from the acid fraction decreased the activity of indoleacetic acid, whereas addition of ether from the neutral fraction was without effect. The values for the auxin activity of the acid fractions of pollen extracts are based on curvatures obtained by testing 8 ml. of the 25 ml. which constitute the acid fraction. The apparent decrease in auxin concentration caused by 8 ml. of the acid fraction of pure ether shaken with tartaric acid varied with the concentration of indoleacetic acid as follows:

Indoleacetic acid, μgm/l	40	60	90
Apparent decrease, μgm/l	10	26	48

As a consequence of these results all determinations of auxin activity in the acid fractions of pollen extracts were based upon readings on a special activity curve constructed from data obtained by dropping varying amounts of indoleacetic acid together with 8 ml. of the acid fraction of ether treated as described above.

The fact that it was impossible to construct the corrected activity curve in the same laboratory in which the pollen extracts were tested tends to decrease the accuracy of the absolute values for the

auxin activities in the acid fractions. The figures do, however, show the order of magnitude of these values and will at least serve to indicate their relative height.[2]

Neutral growth substance was assayed as such, without any preceding conversion to acid. Growth-promoting activity of neutral fractions was first expressed as μgm.-"equivalents" of indole acetaldehyde, one μgm.-"equivalent" of indole acetaldehyde being the amount which has the same effect in the *Avena* test as one μgm. of pure indoleacetic acid when tested at such a dilution that the resulting curvatures are $\leqq 15°$ (or with water-cultured test plants, $\leqq 5°$). The number of μgm.-"equivalents" were converted to μgm. upon the assumption that one μgm-"equivalent" has the actual weight of 10 μgm. (**17** and unpublished data).

Growth-retarding activity was determined by dropping a measured amount of extract together with a known amount of pure indoleacetic acid on the same agar disk and measuring the auxin activity of the agar in the *Avena* test. If the auxin activity of the mixture is lower than that obtained with the same amount of indoleacetic acid alone, the difference is taken as an expression of the growth-retarding activity of the extract (**14, 16**). In all cases the extract of 33 mg. of pollen was added to an agar disk weighing 110 mg. and containing 7.7×10^{-3} μgm. of indoleacetic acid. If, for instance, the resulting curvature corresponds to only 2.9×10^{-3} μgm., the apparent decrease in auxin content is 4.8×10^{-3} μgm. (or 14.4×10^{-3} μgm. of indoleacetic acid per 100 mg. pollen).

It should be noted, however, that values obtained in this way are not true quantitative expressions of the amounts of growth-retarding substance(s). The result will be influenced by auxin contained in the pollen extract even if it is present in amounts too small to overcome the effect of the inhibitor(s). Similarly, in determinations of growth-promoting substances, only the balance of growth-promoting and growth-inhibiting substances is recorded. Therefore, the expressions "growth-promoting" and "growth-retarding activity" are used rather than amount of growth-promoting or growth-retarding substance(s).

Results

Yield of pollen

The yield of pollen in the four varieties, as determined by the amount of dry pollen which could be obtained per flower by the method described, is given in table 1. The number of pollen grains per milligram of pollen was determined by counting in a Thoma-Zeiss counting chamber. From the results the number of pollen grains per anther was calculated. The values obtained in this way are minimum values, since perhaps 10–20% of the pollen may have adhered to the flowers and thus have escaped collection. The relative values, however, are probably nearly correct. They show that the diploid, James Grieve, yields 2.6–8.5 times as many pollen grains per anther as

[2] The influence of the inhibiting effect of ether extracts of tartaric acid is greater in the present study than in most of the auxin determinations made previously by the senior writer, because such a large proportion of the pollen extract was required for each test, owing to the low auxin content of the pollen and the difficulty of collecting large quantities of pollen. In the study of the conversion of indole acetaldehyde to indoleacetic acid (**17**) the significant determinations of the auxin activity of the acid fractions were in general made with less than 1 ml. of the extract, in which case the inhibiting effect of the tartaric acid extract is negligible. Tartaric acid was originally chosen because it is very sparingly soluble in ether and buffers well at pH values between 3 and 4. For future experiments a different acid would be desirable. Hydrochloric acid does not impart inhibiting properties to ether. It is difficult, however, to maintain a pH value between 3 and 4 when pure HCl is used.

the triploid varieties. The extremely low yield of pollen in Graastener is in accord with the fact that this variety is known as a very poor pollinator.

Table 2 shows some characteristics of the pollen grains from the different varieties. The weight of one dry pollen grain was calculated from the number of pollen grains per milligram. The figures indicate that the heaviest grains are found among the triploids, although pollen grains from Tompkins King weigh only a little more than grains from James Grieve. To see whether the order of magnitude of these weights was reasonable, the volume of the dry pollen grains was determined by measuring two dimensions of the grains and calculating their volumes as rotation ellipsoids (James Grieve pollen grains are more oblong than grains from the triploids). These volumes (table 2) give specific gravities of the dry pollen grains varying from 1.1 to 1.3; these seem to be reasonable, since the specific gravity of sugars and of cellulose is about 1.4. Measurements of the volumes in distilled water showed that pollen grains from the diploid, James Grieve, swell more than grains from the triploid varieties. The percentage of empty pollen grains (table 2), determined after staining with acetocarmine (**29**), is much higher in the triploids than in James Grieve.

TABLE 1

YIELD OF POLLEN FROM DIFFERENT APPLE VARIETIES

	James Grieve (2n)	Graastener (3n)	Tompkins King (3n)	Belle de Boskoop (3n)
Mg. pollen per flower (=20 anthers)	1.85	0.43	0.42	1.13
No. of grains per mg. pollen (thousands)	69±3.7*	35±4.2*	60±7.8*	43±6.4*
No. of counts	16	10	10	10
No. of pollen grains per anther (thousands)	6.38	0.75	1.26	2.42

* Standard error based on 42 degrees of freedom.

TABLE 2

CHARACTERISTICS OF POLLEN GRAINS FROM DIFFERENT APPLE VARIETIES

	James Grieve (2n)	Graastener (3n)	Tompkins King (3n)	Belle de Boskoop (3n)
Weight of one dry grain, μgm.$\times 10^{-3}$	14.5	28.6	16.7	23.2
Length of dry grains, μ*	45.3±0.59	46.3±1.44	38.4±1.02	47.2±1.43
Breadth of dry grains, μ*	22.3±0.91	29.5±2.02	27.6±1.41	27.6±1.04
Volume of one dry grain, $\mu^3\times 10^3$	12.0	21.3	15.3	18.8
Specific gravity of dry grains	1.2	1.3	1.1	1.2
Length of grains in distilled water, μ*	37.0±0.59	42.0±1.44	38.0±1.02	40.0±1.43
Breadth of grains in distilled water, μ*	32.2±0.91	35.1±2.02	33.2±1.41	35.4±1.04
Volume of one grain in distilled water, $\mu^3\times 10^3$	20.1	27.3	22.0	26.3
Swelling in water	1.7×	1.3×	1.4×	1.4×
Percentage of empty pollen grains	8	35	32	30

* Mean of measurements of 10 grains; standard error based on 18 degrees of freedom. Thickness of cell wall of pollen grains in all varieties approximately 1.4 μ.

POLLEN GERMINATION

The germination experiments (table 3) confirmed the general observation that triploids are much inferior to diploids

with respect to pollen germination. Pollen from young flowers germinated better than pollen from older flowers. Storing the pollen in a desiccator at 6° C. for 12–14 days reduced its germinability considerably. Stored pollen from the three triploid varieties (results not listed in table 3), tested at 21.5%, and 30% sucrose and at pH values of 4.9 and 6.9, did not germinate at all. Addition of 1 or 10 mg. of indoleacetic acid per liter did not promote germination in these varieties, nor did it improve germination in James Grieve. Thirty per cent sucrose and a pH value of 4.9 in general were more favorable for germination than 21.5% sucrose and a pH value of 6.9, although exceptions to this rule were found.

TABLE 3

GERMINATION OF POLLEN GRAINS

Apple variety	Concentration of		pH = 4.9		pH = 6.9	
	Sucrose (%)	Indoleacetic acid (mg/l)	Percentage germination	Length of pollen tube (μ)	Percentage germination	Length of pollen tube (μ)
	Fresh pollen from young flowers					
James Grieve (2n)	21.5	0	92	915	72	625
Belle de Boskoop (3n)	21.5	0	<1	20	6	20
	Fresh pollen from older flowers					
James Grieve (2n)	21.5	0	66	380	13	396
James Grieve (2n)	30	0	64	670	70	550
Graastener (3n)	21.5	0	0	.....	0	.....
Tompkins King (3n)	21.5	0	0	.....	0	.....
Belle de Boskoop (3n)	21.5	0	6	580	0.8	46
Belle de Boskoop (3n)	30	0	<1	46	4.8	125
	Pollen stored in desiccator at +6° C. for 12–14 days					
James Grieve (2n)	21.5	0	25	210	15	89
James Grieve (2n)	30	0	28	500	18	106
James Grieve (2n)	30	0	29	270	.....	.....
James Grieve (2n)	30	1	15	200	.....	.....
James Grieve (2n)	30	10	33	270	.....	.....

Activity of pollen extracts

The growth-promoting and growth-retarding activities of extracts of pollen from the three varieties, James Grieve, Graastener, and Tompkins King, were determined. The activities per 100 mg. of pollen are given in table 4, while table 5 shows the activity per pollen grain. (The number of grains per 100 mg. of pollen differs from one variety to another, as shown in table 1.)

Growth-promoting activity.—Neutral growth substance, which is assumed to be indole acetaldehyde, was found af-

ter 24 hours of extraction in pollen from the triploid varieties but not from the diploid. After 1 week, it was found in the triploid Graastener but not in the others.

The presence of acid auxin could be demonstrated in all extracts after 24 hours and after 1 week of extraction. The acid fraction of extracts collected after 1 hour and after 2 hours did not show any activity when tested directly. When such extracts were dropped on agar together with 7.7×10^{-3} μgm. of indoleacetic

TABLE 4

GROWTH-PROMOTING AND GROWTH-RETARDING ACTIVITY OF POLLEN EXTRACTS. GROWTH-PROMOTING ACTIVITY EXPRESSED AS μGM.$\times 10^{-3}$ OF INDOLE ACETALDEHYDE (NEUTRAL FRACTION) OR INDOLEACETIC ACID (ACID FRACTION) PER 100 MG. OF POLLEN. GROWTH-RETARDING ACTIVITY EXPRESSED AS NUMBER OF μGM.$\times 10^{-3}$ OF INDOLEACETIC ACID THE ACTIVITY OF WHICH IS COMPENSATED BY EXTRACT OF 100 MG. OF POLLEN

Apple variety	Type of activity	1st hour		2d hour		3d–24th hour		25th–168th hour		Total	
		Neutral fraction	Acid fraction*	Neutral fraction	Acid fraction*	Neutral fraction	Acid fraction*	Neutral fraction	Acid fraction*	Neutral fraction	Acid fraction
James Grieve (2n)	Promoting	0	(0) 16	0	(0) 6	0	12 (0)	0	29 (35)	0	63
	Retarding	14	.....	3	.....	15	.....	1	.....	33	.....
Graastener (3n)	Promoting	0	(0) 12	0	(0) 11	86	18 (26)	76	42 (39)	162	83
	Retarding	12	.....	11	.....	4	.....	7	.....	34	.....
Tompkins King (3n)	Promoting	0	(0) 11	0	(0) 5	90	45 (21)	0	65 (41)	90	126
	Retarding	10	.....	10	.....	2	.....	1	.....	23	.....

* Growth-promoting activity of acid fractions: first figure, direct method; second figure, differential method (see text). Figures in parentheses not used for further calculations.

TABLE 5

GROWTH-PROMOTING AND GROWTH-RETARDING ACTIVITY OF POLLEN EXTRACTS. GROWTH-PROMOTING ACTIVITY EXPRESSED AS μGM.$\times 10^{-9}$ OF INDOLE ACETALDEHYDE (NEUTRAL FRACTION) OR INDOLEACETIC ACID (ACID FRACTION) PER POLLEN GRAIN. GROWTH-RETARDING ACTIVITY EXPRESSED AS NUMBER OF μGM.$\times 10^{-9}$ OF INDOLEACETIC ACID THE ACTIVITY OF WHICH IS COMPENSATED BY EXTRACT OF ONE POLLEN GRAIN

Apple variety	Type of activity	1st hour		2d hour		3d–24th hour		25th–168th hour		Total	
		Neutral fraction	Acid fraction	Neutral fraction	Acid fraction	Neutral fraction	Acid fraction	Neutral fraction	Acid fraction	Neutral fraction	Acid fraction
James Grieve (2n)	Promoting	0	2.3	0	0.9	0	1.7	0	4.2	0	9.1
	Retarding	2.1	.....	0.4	.....	2.2	.....	0.1	.....	4.8	.....
Graastener (3n)	Promoting	0	3.4	0	3.1	24	5.1	22	12.0	46	23.6
	Retarding	3.4	.....	3.1	.....	1.1	.....	2.0	.....	9.6	.....
Tompkins King (3n)	Promoting	0	1.8	0	0.8	15	7.5	0	10.9	15	21.0
	Retarding	1.7	.....	1.6	.....	0.3	.....	0.2	.....	3.8	.....

acid in order to test for growth inhibitors (cf. section on materials and methods), it appeared, however, that the resulting curvatures were larger than those induced by the same amount of indoleacetic acid plus 8 ml. of ether which had been shaken with tartaric acid. These results indicate that the extracts had some auxin activity, although not enough to produce a concentration of auxin in the agar disk which was higher than the threshold value of the *Avena* test. The added amount of indoleacetic acid, 7.7×10^{-3} μgm., was subtracted from the total auxin activity of the mixture. The balance was taken as a measure of the auxin activity of the extract. The results obtained by this differential method and by the direct determinations are both entered in table 4. The direct method must be considered more accurate than the differential method. For calculation of the auxin activity per pollen grain (table 5), therefore, the values obtained by the direct method were used except where this method failed (1st and 2d hour).

The sum of the growth-promoting activities obtained by successive extractions is lower in James Grieve than in the other varieties, both per 100 mg. of pollen and per pollen grain.

GROWTH-RETARDING ACTIVITY.—Substances which retarded growth were present in all the neutral fractions of the extracts. The growth-retarding activity in general was higher after 1 or 2 hours of extraction than after 24 hours or 1 week. It should be remembered in this connection that extractable substances originally present in the pollen or produced during the first periods of extraction were removed to a large extent before the later extracts were collected.

The neutral extracts had the ability to decrease the activity of added indoleacetic acid even in cases where they showed growth-promoting activity when tested alone. In these cases the values for both growth-promoting and growth-retarding activity must be considered minimum values.

No consistent correlation between the pollinating value and the content of growth-retarding substances of the pollen is apparent.

It is probable that the active substances are present in the normal pollen grains only and not in the empty ones (table 2). Even when the growth-retarding activities are calculated per normal grain only, however, no consistent trend in their occurrence is observable.

GROWTH-PROMOTING ACTIVITY OF ANTHER EXTRACTS

The growth-promoting activities of extracts from entire anthers of the four varieties are listed in table 6. The values per pollen grain were obtained by utilizing the results, given in table 1, of counts of the number of pollen grains per anther. The anthers were extracted for 24 hours with two changes of ether. In order to compare the growth-promoting activities obtained by extraction of entire anthers and of stored pollen, the sums of growth-promoting activities obtained during the first 24 hours of extraction of stored pollen are included in the table. These figures show that the acid fractions of the extracts at 24 hours had approximately the same growth-promoting activity per pollen grain as the extracts of stored pollen except in the case of Tompkins King. Extracts of the stored pollen of this variety showed 47% less auxin activity per pollen grain than extracts of the anthers. If the neutral fractions are compared in the same manner, it appears that the stored pollen yielded much less than the anthers. The difference is too great to be explained by losses in the collection of pollen from the dry flowers. It must be

assumed either that some growth substance is contained in the wall of the anther or that a loss of growth substance has occurred during drying and storage of the pollen. The latter possibility seems to be the more probable. The loss amounts to 96–100% of the neutral growth-promoting activity. THIMANN and SKOOG (26) found a loss of extractable auxin (probably acid + neutral) by drying of *Lemna* fronds, *Avena* roots, and *Phaseolus* root nodules which amounted to 86, 55, and 57%, respectively. In *Avena* coleoptiles no such decrease occurred through drying. GORDON and NIEVA (5) reported a 100% decrease of the amount of extractable indole acetaldehyde by drying pineapple leaves at 80° C.

TABLE 6

GROWTH-PROMOTING ACTIVITY OF ANTHER EXTRACTS EXPRESSED AS CORRESPONDING AMOUNTS OF INDOLE ACETALDEHYDE (NEUTRAL FRACTIONS) OR INDOLEACETIC ACID (ACID FRACTIONS) ANTHERS EXTRACTED FOR 24 HOURS WITH TWO CHANGES OF ETHER

APPLE VARIETY	μGM. $\times 10^{-1}$ PER 100 MG. ANTHERS		μGM. $\times 10^{-5}$ PER ANTHER		μGM. $\times 10^{-9}$ PER POLLEN GRAIN*	
	Neutral fraction	Acid fraction	Neutral fraction	Acid fraction	Neutral fraction	Acid fraction
James Grieve (2*n*)	20	1.8	280	25	44 (0)	3.9 (4.0)
Graastener (3*n*)	24	0.5	460	10	614 (24)	13.3 (11.6)
Tompkins King (3*n*)	36	1.7	500	24	306 (15)	10.0 (10.1)
Belle de Boskoop (3*n*)	18	2.0	320	38	132	15.7

* Figures in parentheses represent sums of growth-promoting activities attained during first 21 hours of extraction of stored pollen (table 5).

TABLE 7

WEIGHT OF MATERIAL EXTRACTED FROM 100 MG. OF JAMES GRIEVE POLLEN

	1ST HOUR		2D HOUR		3D–24TH HOUR		25TH–168TH HOUR	
	Neutral fraction	Acid fraction	Neutral fraction	Acid fraction	Neutral fraction	Acid fraction	Neutral fraction	Acid fraction
Total weight of extract (mg.)	1.91	1.11	3.16	2.85	0.33	2.27	0.47	1.42
Weight of impurities in ether (mg.)*	0.10	1.03	0.10	1.03	0.10	1.03	0.10	1.03
Weight of solids originating from pollen (mg.)	1.81	0.08	3.06	1.82	0.23	1.24	0.37	0.39

* Weight of "impurities" determined after shaking pure ether with sodium bicarbonate solution (neutral fraction) and with solution of tartaric acid (acid fraction) just as in fractionation of pollen extracts. Most of solids in acid fraction are probably tartaric acid.

WEIGHT OF EXTRACTED MATERIAL

The weight of the material extracted from James Grieve pollen grains was determined by evaporating the extracts from 100 mg. of pollen and drying the residue in a desiccator over $CaCl_2$ for 2–4 hours. No further reduction of the weight was obtained by drying for 2 additional hours at 60° C. The residues were weighed on a Kuhlmann microbalance sensitive to 0.001 mg. (table 7). From

this table and table 4 it appears that the purest extract of neutral growth inhibitor(s) is obtained during the 3d to 24th hour of extraction. In this case 230 μgm. of extracted material were capable of compensating the growth-promoting activity of 15×10^{-3} μgm. of indoleacetic acid in the *Avena* test. On the basis of previous experiments with anemonin and parasorbic acid (**16**) it may be concluded from these figures that the pollen grains contain a neutral inhibitor which is more active than either of these two substances.

Discussion

Assuming that the growth-retarding activity as determined by the method employed does actually indicate the potency of the inhibitor(s) in counteracting the growth-promoting substances, it appears from table 5 that there is practically no excess of growth-promoting over growth-retarding activity in the 1st- and 2d-hour extracts. Such an excess, however, develops later, and the sums of the activities after 1 week show a considerable excess of growth-promoting activity. In many plant materials auxin is known to be released enzymatically from a bound state during extraction with "wet" ether at room temperature. Such a release of auxin is apparently also going on during the extraction of the pollen. If inhibitors are also released from a bound state (which is entirely possible), it must be assumed that more growth-promoting substance than inhibitor is released per unit of time. Under such circumstances the biological activities of the extracts found after different periods of extraction and the sums of them are not caused by substances present at the beginning of the extraction. The measured activities do, however, indicate the potency of the pollen grains in releasing active substances. The observed differences in biological activity of the extracts may indicate differences in content of the enzymes involved in the release of active substances or in amounts of precursors present.

The results of the determinations of auxin and inhibitors do not bear any obvious relationship to the germination percentage of the pollen. James Grieve pollen, which showed the highest percentage of germination, has a lower growth-promoting activity per pollen grain than pollen from any of the other varieties. There is no clear relationship between growth-retarding activity and germinability. The total excess of growth-promoting over growth-retarding activity is considerably less in James Grieve pollen than in pollen from the triploids. These results, however, are in accord with the observation that pollen germination was in no case improved by artificial addition of indoleacetic acid.

Even though the determinations of growth-promoting and growth-retarding substances reported here do not contribute to an understanding of the differences in pollinating value of the diploid and triploid varieties, they do, on the other hand, demonstrate the need for adequate methods for the separation and determination of these two groups of substances. The cress-root test of MOEWUS (**19, 20**) might prove to be very useful for such determinations.

It may be said in conclusion that the triploid apple varieties dealt with in the present study, like most other triploids, owe their low pollinating value in part to the low number of pollen grains they produce per anther and in part to the low germination percentage of their pollen. In addition, many of the pollen grains which do germinate will probably lack the capacity of fertilizing the ovules. The

low viability of pollen from triploids is generally explained as a consequence of a lack of "genic balance" of the pollen grains. It would be desirable to analyze this unbalanced condition biochemically. Such an analysis, however, encounters the difficulty that the genic composition of the pollen grains is far from being uniform. The chromosome number of the pollen grains may, theoretically, be anywhere from n to $2n$, although the large majority have chromosome numbers around $1\frac{1}{2}n$. The actual combination of the genes, however, may vary from one grain to another even when the chromosome number of the grains is the same. The low viability of pollen grains in the unbalanced condition is probably caused by an under- or overproduction of certain substances. It must be assumed, however, that the nature and quantity of the significant substances vary with the genic combination from grain to grain.

In view of these considerations it is perhaps not surprising that no correlation was found between the content of growth-regulators and the pollinating value of such a heterogeneous material as pollen from triploids.

Summary

1. The material for the present study was pollen from one diploid and two (in some cases three) triploid apple varieties.

2. In the triploid varieties the number of pollen grains per anther was 72–88% lower than in the diploid.

3. The percentage of germination was much higher in pollen from the diploid variety than in pollen from the triploids. In no case was germination improved by the addition of indoleacetic acid.

4. A neutral and an acid growth-promoting substance and a neutral growth-inhibitor (*Avena* test) were obtained by ether extractions of dried pollen. (Extracts were collected for assay from a given sample of pollen after 1, 2, 24, and 168 hours, fresh ether being added each time.)

5. After 1 and 2 hours the growth-promoting and the growth-retarding activities of the extracts were almost equal (table 5). Later on, however, an excess of growth-promoting activity developed. The total excess of growth-promoting over growth-retarding activity was considerably less in pollen from the diploid than in pollen from the triploids.

6. The results of the determinations of growth-promoting and growth-retarding substances in the pollen grains do not explain the differences in germinability and pollinating value of pollen from the diploid varieties.

7. From determinations of the weight of the material extracted from the pollen grains, it is concluded that the growth inhibitor is more active in the *Avena* test than anemonin and parasorbic acid.

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A LEAF-REPRESSION METHOD FOR EVALUATION OF FORMATIVE ACTIVITY OF PLANT GROWTH-REGULATING CHEMICALS

JAMES W. BROWN AND ROBERT L. WEINTRAUB

Introduction

Beginning with the now classical *Avena*-coleoptile-curvature method of WENT (7), several techniques have been employed for measurement of plant responses to growth-regulating chemicals. Experience with the various tests and with the ever increasing assemblage of effective compounds has demonstrated that no single method is adequate for measuring, or even demonstrating, the manifold effects which may be induced by these substances. Some compounds may exhibit high efficacy in eliciting certain types of response but little or no activity in others; similar differences may be found even among various techniques which measure, presumably, the same kind of response.

Such discrepancies are doubtless due in part to technical difficulty in distinguishing between what may be termed "intrinsic activity" and "apparent activity" of a substance. If it is assumed that growth-regulators are effective by virtue of some chemical reaction in which they participate, then the intrinsic activity would be a measure of the effectiveness of such participation. In practice, what is actually measured is an over-all activity which may be the resultant of such ancillary properties as penetrability of the compound into the plant, solubility in the plant fluids or protoplasm, transportability of the substance within the plant, and rate of inactivation of the material *in vivo*. In addition, of course, the intrinsic activity of a particular compound may vary in different types of response. Furthermore, various species of plants may exhibit quite dissimilar responsiveness to a single substance.

Thus far, the only methods developed beyond a semiquantitative stage are those which depend upon stimulation of cell extension in shoots, upon inhibition of root elongation, or upon stimulation of ovary development. The present report describes a technique for measuring a so-called "formative" effect as manifested by repression of expansion of immature leaves.

Modifications in form and venation of developing leaves which resulted from application of β-naphthoxyacetic acid and certain of its derivatives were observed and designated as "formative" effects by ZIMMERMAN (8) and by ZIMMERMAN and HITCHCOCK (11). Later, various phenoxy and benzoic acids were found to induce similar effects (9, 10, 12). BEAL (1) proposed the term "telemorphic" to include responses of this type occurring in organs other than that to which the compound was applied. Morphological and histological studies of the leaf modifications have been reported by BURTON (2) and by WATSON (6). Formative effects are generally accompanied by diminished development of leaves and stems, and observation suggests that, in some instances, at least, the formative potency of a growth-regulator may be closely correlated with its herbicidal effectiveness.

Development of test

The single-droplet water test on kidney bean, introduced by MITCHELL and HAMNER (4) and employed extensively by THOMPSON *et al.* (5) for comparing inhibitory activities of a long series of compounds, appeared promising of development into a quantitative assay method. This test consists of placing a small drop of growth-regulator solution upon one primary leaf of a kidney-bean seedling and measuring the fresh weight of the subsequently developed shoot tissue. In the experiments of THOMPSON *et al.* (5) the test substances were applied at a uniform dose of 4 μgm. per plant, in aqueous solution containing Carbowax 1500; no data were reported at other levels.

Further development of this method was attempted along three lines: (*a*) establishment of the quantitative nature of the response; (*b*) increase in sensitivity of the test; and (*c*) elimination or minimization of penetration and translocation as influential factors.

In the present experiments 2,4-dichlorophenoxyacetic acid (2,4-D) has been employed as the standard test substance. Twelve to twenty-four replicate plants were used for each treatment. The percentage repression of development as compared with a control receiving no growth-regulator was used as a measure of response. The principal results of the developmental experimentation may be summarized briefly.

BEAN VARIETY.—The Black Valentine variety of *Phaseolus vulgaris* was found to be slightly more sensitive than Red Kidney and has been employed in all the subsequently described experiments. The Red Kidney bean also is suitable for this test, however, and the method doubtless could be applied to other species, some of which might be considerably more responsive. Later trials with Lincoln and Black Wilson varieties of soybean indicated that this species is only about one-third as sensitive as the varieties of garden bean tested.

SITE OF APPLICATION OF GROWTH-REGULATOR.—A pronounced increase in the inhibitory efficacy of a particular dose was achieved by placing the droplet upon the terminal bud or upon the internode just beneath the bud rather than upon a primary leaf blade; for equal repressions, only one-tenth to one-twentieth as great a dose need be applied to the bud, presumably because of the virtual elimination of translocation and dilution in the tissues as factors in the response. Because translocation is known to be markedly influenced by environmental conditions, probably because of the effect of the latter upon the carbohydrate status of the leaf, its elimination might be expected, furthermore, to bring about a decrease in variation among tests performed at different times in uncontrolled environments.

Application of the solution directly to the bud proved to be slightly preferable to treatment of the internode in the technique ultimately evolved, and all the subsequently described tests were made by this method. As will be discussed below, however, there may be circumstances in which applications to the internode are desirable.

SIZE OF DROPLET.—It was found beneficial to use a droplet smaller than that employed in the earlier work. With the commercially available pipettes the smallest practicable volume is 0.005 ml. This is delivered from a 0.1-ml. serological pipette, subdivided into 0.001-ml. intervals, by allowing the drop to form at the pipette tip and touching it to the desired site. Delivery of these small drops is facilitated by using pipettes with the tips somewhat drawn out. By attaching a

0.25-ml. tuberculin-type syringe to the pipette with a short length of heavy rubber tubing, more precise control can be obtained than with the fingertip. Commercial pipettes of good quality have been found sufficiently accurate to render calibration unnecessary.

SOLVENT FOR GROWTH-REGULATOR.—Certain organic liquids were found to be preferable to water as the solvent for two reasons: (*a*) greater facility in delivering small volumes from the pipette and (*b*) markedly greater effectiveness of a specific dose of growth-regulator, presumably as a consequence of the much more rapid absorption of solution by the tissue at the site of application and hence more complete entry of the growth-regulator. Of a number of solvents tested, 95% ethyl alcohol was found most suitable for beans, because alone it had no repressive effect upon development. Acetone and Deobase (a petroleum fraction) also are usable although not entirely innocuous. Fuel oil is less satisfactory. Chloroform, benzene, diethyl ether, and dioxane are unsuitable because they cause death of the treated buds.

A further advantage of great importance accrues through the use of organic solvents inasmuch as many of the compounds which it may be desired to test are relatively insoluble in water—a circumstance that has seriously hampered many previous studies. For some substances that are insufficiently soluble in pure alcohol or acetone, aqueous mixtures containing 50–75% of these solvents may be employed advantageously with relatively little diminution of effectiveness.

The use of water-diluted solvents may be necessary also with other species more sensitive to the pure solvents than are beans. In a limited survey, the buds of cucumber, squash, soybean, and brown-eyed pea seedlings were injured by 95% alcohol, whereas those of castor bean were not. Alcohol at 75% concentration was satisfactory for soybean and brown-eyed pea.

Some further enhancement of the potency of growth-regulators in alcoholic solution is obtained through the incorporation of a surface-active agent in the solution. Of a number of such substances tested, Tween-20 (a polyoxy-alkalene derivative of sorbitan monolaurate manufactured by Atlas Powder Co.) proved to be best, in that its use results in the greatest increase in activity, while the detergent itself, in alcoholic solution, is inert as a formative agent when properly applied to buds of beans of the size specified below.

Ninety-five per cent ethyl alcohol containing 1% of Tween-20 is a satisfactory solvent for the great majority of the compounds thus far investigated and has been employed routinely. Comparison was made between the first trifoliolate leaves developing from untreated buds and from those treated with this solvent. In a series of twenty-two tests, extending over a period of 6 months, and each comprising sixteen plants of either category, the mean area of these leaves from the solvent-treated plants was $94.9 \pm 2.7\%$ and their mean fresh weight $99.0 \pm 2.2\%$ that of the untreated. The repression caused by the solvent is not a formative effect but rather is a nonspecific injury owing to killing of certain cells of the embryonic leaf, particularly those which give rise to the apical portions of the leaflets and especially of the central leaflet. Solvent injury is ordinarily manifested by a rounding of the normally acuminate leaflets; occasionally, more severe injury results in emarginate or obcordate leaflets (fig. 6 and discussion below). The injury appears to be greater if the solu-

tion is applied directly to the tip of the bud; the practice was therefore adopted of placing the drop at the base of the bud. With quite small buds it is sometimes difficult to confine the drop to this region, so that leaves from such buds are more likely to exhibit abnormal development. Because the solvent effect could not be eliminated entirely, solvent-treated plants have been employed as controls in each series of tests with a particular planting of beans.

CRITERIA OF RESPONSE.—Response to formative growth-regulators can be measured by the degree of repression of the various organs of the shoot which develop subsequent to application. A study of the developmental changes in size and fresh weight of internodes and leaves indicated that the most pronounced and reproducible effect was upon the expansion of the first trifoliolate leaf. Results illustrating the relative sensitivity of various criteria investigated are presented in figure 1.

Leaf area was determined either by direct measurement with a photometric instrument constructed for the purpose or by planimetering shadow prints made on reflex copy paper with the aid of a contact printer. Results obtained by the two methods were in close agreement. The direct method has the advantage of furnishing results with a minimum of delay, whereas the indirect technique provides a permanent photographic record of the morphological peculiarities of the leaves, which has proved useful in classification of the different types of effects discussed below.

STAGE OF DEVELOPMENT OF TEST PLANT.—The responsiveness of each successive leaf to growth-regulator appears to be closely correlated with its developmental state at time of application. The vulnerability of the developing leaf reaches a maximum when it is a few millimeters long and thereafter diminishes quite rapidly (see also WATSON [6]). For the first trifoliolate leaf the stage of maximum sensitivity is attained just prior to its unfolding from the bud. Ordinarily the subjacent internode commences to elongate before the bud opens, so that length of the bud stalk furnishes a convenient gauge of the status of the bud. This relationship may be modified by environmental conditions, however, high-

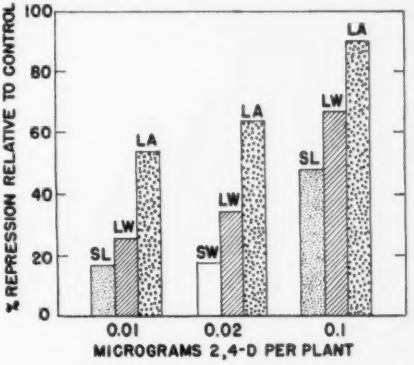


FIG. 1.—Comparison of various criteria of effectiveness of formative growth-regulators. *SL*, stem length; *SW*, fresh weight of shoot above primary leaf node; *LW*, fresh weight of first trifoliolate leaf; *LA*, area of first trifoliolate leaf.

light intensity tending to suppress internodal elongation. The optimal bud stage is usually found when the internode is 3–7 mm. long. For the greatest uniformity of response it is essential to employ plants in which the trifoliolate leaves are at a similar stage of development. Failure to judge correctly the developmental status of the very young leaf by its outward appearance is believed to be responsible for much of the variation found among replicates.

Figure 2 portrays a typical plant at the stage suitable for application, together with control and treated plants at the conclusion of the test period.

In practice, three seeds are planted per 4-inch pot in a soil-sand-peat mixture. When the plants develop to the desired size in the greenhouse, having been thinned a day earlier to one per pot, two-thirds to three-fourths of the population commonly falls within the limits prescribed. During spring and summer the plants attain suitable size on the sixth or seventh day after planting; during winter, or if a protracted period of cloudy weather occurs after emergence, an additional day may be required. The use of bottom heat to maintain soil temperature at 26°–28° C. proved advantageous in winter.

Whether the cotyledons, which are not quite exhausted at the stage selected for treatment, are removed from the plant or not has relatively little influence upon the subsequent response.

ENVIRONMENT.—Tests were conducted in a greenhouse and in an artificially lighted room with temperature and humidity controlled, to ascertain whether the sensitivity of the response could be increased appreciably by manipulation of the environment. All the test plants were cultured in the greenhouse, as previously described, until they reached the proper size for treatment, at which time they were either transferred to the controlled room or retained in the greenhouse. Under the most favorable controlled conditions provided, there was no consistently greater sensitivity than in the greenhouse, although there was some indication that reproducibility among replicate plants and among successive tests might be slightly better. The controlled conditions which yielded results similar to those in the greenhouse were: light intensity, 800 foot-candles (furnished by Daylight fluorescent

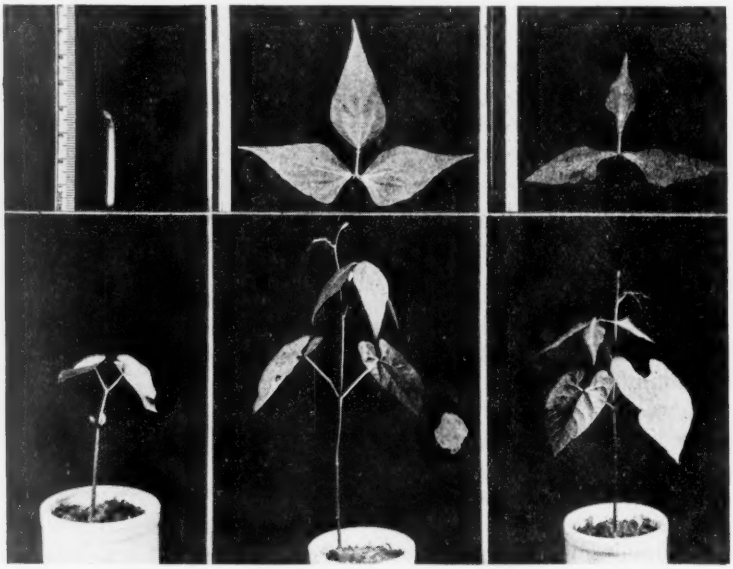


FIG. 2.—Typical plants (*lower row*) and first trifoliolate leaves (*upper row*). *Left*, at stage treated; *center* solvent control at harvest; *right*, 2,4-D–treated at harvest.

lamps); photoperiod, 14–16 hours; day temperature, 25° ± 1° C.; night temperature, 23° ± 1° C.; relative humidity, 50–70%.

DURATION OF TEST.—Figure 3 illustrates an experiment in which the relation of response to the interval between application and measurement was studied; similar results were obtained in several others. From the fifth to the thirteenth day after application, the repression of leaf expansion, as compared with controls of corresponding ages, is substantially constant. Hence the duration of the test is not critical within these limits. In practice the leaves are harvested when they are judged to have attained about two-thirds of maximum size; this requires 6–7 days during the more favorable half of the year and a somewhat longer time in winter.

Sensitivity and reproducibility of test

The relation between quantity of 2,4-dichlorophenoxyacetic acid (2,4-D) applied to the plant and resulting repression of leaf development is shown in figure 4. The percentage repression, which is calculated as 100 × (control minus treated)/control, is closely proportional to the logarithm of the dose over an approximately twenty-fold range. The marked increase in thickness of the affected leaves with increasing dosages is

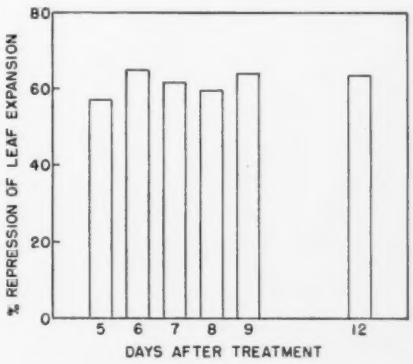


FIG. 3.—Repression of leaf expansion, as compared with controls of corresponding ages, at various intervals following application of 0.025 μgm. of 2,4-D to bud. Each bar represents ratio of 16 treated and 16 untreated plants.

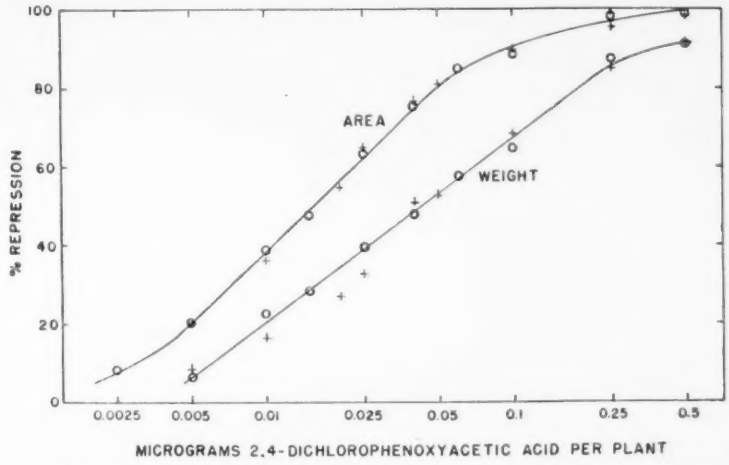
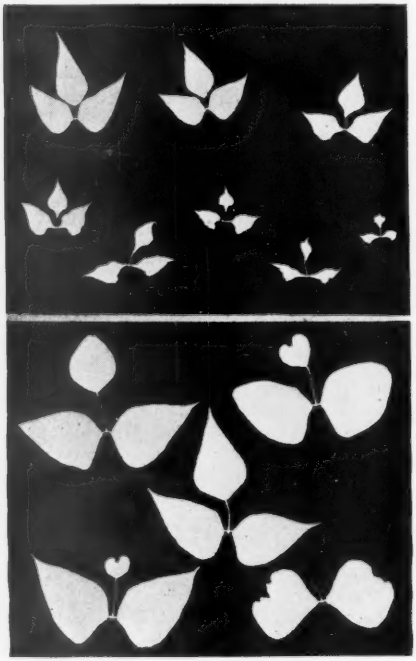


FIG. 4.—Relation between dose of growth-regulator and repression of development of first trifoliolate leaf of Black Valentine bean. Curves drawn through data (circles, 24 plants per dose) from greenhouse experiment using bud application. Crosses represent means of three experiments in controlled room using bud-stalk application (12–36 plants per dose).

reflected in the greater slope of the curve for area repression than of that for weight repression. These curves show a striking similarity to that for stimulation of ovary development (3). Typical leaves are illustrated in figure 5.

For assay purposes the dosage should be chosen to give effects within the limits of 20 and 80%. The repression of weight increase, although a somewhat less sensitive and less precise measure of response, is nevertheless usable and, where some precision can be sacrificed, affords the advantages of being much more quickly and easily determinable than the area response.



FIGS. 5, 6.—Fig. 5 (*above*), representative leaves from plants receiving various doses of 2,4-D. *Left to right, upper row:* 0, 0.005, 0.01; *lower row:* 0.02, 0.025, 0.04, 0.05, 0.1 μgm. Fig. 6 (*below*), types of nonformative injury produced by application of various compounds to embryonic leaf. Untreated leaf at center.

In table 1 are listed the results of a series of greenhouse tests performed during a 3-month period, in autumn and winter, which included many days of very low light intensity. A standard dose of 0.015 μgm. of 2,4-D, which corresponds to 48.9% repression of area in the curve of figure 4, was used throughout. The data show that the *percentage* repression is fairly constant in successive tests, even though the actual size of the leaves covers a more than threefold range owing

TABLE 1

REPRESSION OF LEAF EXPANSION IN SERIES OF GREENHOUSE TESTS. DOSE WAS 0.015 μGM. OF 2,4-D PER PLANT. SIXTEEN REPLICATES EACH FOR TREATED AND CONTROL PLANTS IN EVERY TEST

Test period	Mean area per treated leaf (cm^2)	% repression of area and standard error*
Nov. 10–18, 1948	14.8	41.1 ± 8.7
Nov. 15–24	16.9	45.3 ± 12.6
Nov. 16–20	41.1	49.3 ± 5.7
Nov. 18–29	32.2	42.3 ± 5.9
Dec. 2–13	26.8	49.4 ± 4.9
Dec. 8–17	16.3	52.9 ± 3.8
Dec. 10–20	23.7	57.8 ± 2.8
Dec. 16–27	29.6	40.6 ± 5.3
Dec. 21–30	16.3	46.7 ± 5.0
Dec. 22–31	14.0	54.3 ± 6.4
Dec. 28–Jan. 6	13.2	48.6 ± 4.3
Dec. 29–Jan. 10	43.2	28.1 ± 5.8†
Jan. 5–17	46.8	31.1 ± 5.0†
Jan. 7–17	29.2	48.9 ± 3.2
Jan. 13–24	30.2	51.0 ± 2.5
Jan. 19–31	27.3	51.4 ± 5.3
Jan. 20–31	21.4	50.3 ± 3.7
Jan. 25–Feb. 4	20.9	45.7 ± 4.9
Jan. 26–Feb. 7	30.3	33.6 ± 5.4
Feb. 14–23	28.9	49.7 ± 6.6
Feb. 15–23	21.1	54.1 ± 8.3
Feb. 16–25	27.4	46.8 ± 5.7
Mean		48.0 ± 5.6

* Standard error of percentage repression

$$= 100\sqrt{\left(\frac{m_T}{m_U}\right)^2\left[\left(\frac{s_T}{m_T}\right)^2+\left(\frac{s_U}{m_U}\right)^2\right]},$$

where m_T = mean area of 16 treated replicates

m_U = mean area of 16 solvent replicates

s_T = standard error of m_T

s_U = standard error of m_U.

† Greenhouse temperature fell to low level during part of these tests. Excluded from mean.

to variations in environmental conditions and culture period. This high degree of reproducibility, which has been found also with a number of other growth-regulators, permits expression of the potency in absolute units as contrasted with the single-droplet water test on kidney bean in which ratings are made relative to the activity of 2,4-D. In comparing various compounds, it has been found convenient to employ the following terms:

Leaf area repression activity (LARA) = reciprocal of dose in μgm. which produces 50% repression of area

Leaf weight repression activity (LWRA) = reciprocal of dose in μgm. which produces 50% repression of fresh weight

Molar leaf area repression activity (MOLARA) and *molar leaf weight repression activity* (MOLWRA) = LARA and LWRA, respectively, multiplied by the molecular weight.

Suggested applications

The leaf-repression method would appear to be useful in a number of applications. Some of these, which are being investigated, may be discussed briefly; more detailed accounts will be reported separately.

This technique affords a more quantitative means of comparing the formative activities of diverse substances than has heretofore been available. In practice a sufficient number of doses are tested to determine the dosage-response curve for each growth-regulator. Hence, comparison can be made among doses of various substances which produce a standard level of response (50%) rather than among degrees of response elicited by a fixed dose. This permits the evaluation of compounds of high and of low activity with equal precision. Among a considerable variety of compounds tested, the LARA has been found to cover more than a thousandfold range.

Although the macroscopic morphological effects induced by diverse compounds appear much alike, there are certain, as yet incompletely explored, dissimilarities in response which suggest that differences may exist in type, and perhaps mechanism, as well as in degree of formative activity. In tests of this kind it is imperative to distinguish between the genuine *formative effect* and *nonspecific injury* of the type described above as sometimes caused by the solvent. Many substances which exhibit little or no formative activity are capable, particularly at the high doses which must be tested, of causing severe repression of development, even to the extent of complete suppression of the lamina. A series of nonspecific effects of varying severity is illustrated in figure 6.

The leaf-repression method, perhaps with some modification in particular cases, may be employed in comparing the responsiveness of various plant species and varieties. As mentioned above, preliminary results indicate that the sensitivity of Lincoln and Black Wilson soybeans to 2,4-D is about one-third that of *Phaseolus vulgaris* var. Black Valentine.

The method, even though not specific, is suitable for assay of small quantities of many exogenous growth-regulators and may be especially useful for this purpose pending the development of more satisfactory physical, chemical, or biological techniques.

Owing to the wide spread of activity among various growth-regulators, it is possible, in certain combinations, to determine one active compound in admixture with others. A prerequisite of such a use is the demonstration that neither synergy nor antagonism exists among the components.

The assay of exogenous growth-regulating substances in plant extracts, exudates, and washings also is feasible but

will require development of appropriate procedures for quantitative extraction. As the LARA of 2,4-D is more than one hundred times that of indole-3-acetic acid, it is unlikely that the latter, in the amounts likely to be present in plants, would interfere with the determination of the former.

Information concerning the relation of penetrability and transportability to molecular structure and to environmental and other factors may be derived from measurement of activities as revealed by the bud-application technique, which minimizes entry and transport as complications, in conjunction with other procedures—such as the single-droplet water test on kidney bean—in which these processes play a considerable part. Techniques similar to the single-droplet water test on kidney bean, in which application of growth-regulator is made to well-developed leaves and which are very useful for many physiological studies, may be improved by utilizing leaf repression as a measure of response; as already noted, the sensitivity to leaf application is considerably lower than with bud application.

Summary

A technique for measuring quantitatively a formative effect of exogenous chemical growth-regulators is described. The method, which is based on the repression of leaf development subsequent to application of growth-regulator to the bud, exhibits a rather high degree of sensitivity and reproducibility. A number of uses are discussed.

The authors wish to express their appreciation of the assistance of JEAN C. NICKERSON, ROBERT A. PETERS, KATHARINE N. TAYLOR, ARTHUR THRONE, EDWIN L. WILSON, and JOHN N. YEATMAN with certain of the experiments; of the co-operation of the Photographic Section, Camp Detrick, in preparation of the leaf prints; and of the advice of Dr. CLIFFORD L. MALONEY in connection with the statistical analyses.

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ARTIFICIAL DIOECISM IN RELATION TO PHYSIOLOGICAL ONTOGENY OF THE GHERKIN, CUCUMIS ANGURIA

WAYNE C. HALL

Introduction

Most contemporary studies of physiological ontogeny have indicated certain common and characteristic rhythms in the growth and developmental cycles of herbaceous plants. The influence of sexual processes upon vegetative vigor has been stressed repeatedly (**2, 6, 10, 12, 13, 15, 21, 22, 25, 30, 35, 36,** and others). It is generally accepted that fluctuations in the vegetative growth rate subsequent to the initiation of reproductive structures are, in part or wholly, to be attributed to a stimulation or inhibition associated with development of flower, fruit, and seed.

In published results of studies with the gherkin (**17, 18**) differences in growth and developmental responses were noted between normal flowering controls and emasculated plants subjected to different levels of nitrogen supply and daylength. The present more extensive investigation was conducted concurrently with those experiments to determine whether the variable responses resulted from sexual modification of plants or from other factors. This paper, however, reports the results on plants grown only under high nitrogen, long-day conditions. The monoecious habit of the gherkin readily permits experimental elimination of flowers of either sex and thus provides a means of identifying morphological and physiological effects possibly associated with staminate and pistillate organs. Recognition and interpretation of these effects are greatly facilitated by the use of a monoecious species, as the plant can be completely deflowered, rendered unisexual, or left unaltered as desired, contrasted to a species of the dioecious type.

Experimental procedure

Seeds of the small gherkin were germinated in flats of sand. Transplantings were made 7 days following germination into quartz-gravel, aerated, standing nutrient cultures, which were completely drained and replenished weekly. The plants were then grown to maturity as previously described (**17, 18**). At transplanting the plants were divided into four experimental series as indicated in table 1, and a uniform procedure of bud, flower, and fruit removal was followed throughout the experiment. Flower buds were removed daily as soon as they could be detected with the aid of a hand lens. To insure a high degree of fertilization, hand pollinations were made in the appropriate series. From preliminary work with the gherkin the approximate time of syngamy was identified microscopically in normal flowering plants. It was also noted that fruit enlargement did not

TABLE 1

PLAN OF EXPERIMENTATION
(Twenty plants to a series)

Series	Experimental variables (sexual modifications of plants)
1	♂ Fls. and ♀ fls. (sexually intact controls)
2	♂ Fls. removed, ♀ fls. unpollinated
3	♂ Fls. removed, ♀ fls. pollinated, fruits removed
4	♂ Fls. intact, ♀ fls. removed

occur unless fertilization had taken place. Therefore the beginning of fruit enlargement was used as a criterion that syngamy had occurred in series 3, and fruits were removed promptly as soon as such enlargement was discernible. Unfortunately, the gherkin is a species with indeterminate flowering, and blossoming continues for a considerable time once initiated. Therefore, the developmental phases recognized in this paper are defined on the basis of age in days when these phases were first observable (either microscopically or macroscopically) in the normal flowering controls of series 1. Thus, the vegetative phase is from 0 to 29 days of age; budding (both staminate and pistillate) is from 29 to 43 days of age; anthesis from 43 to 85 days of age; and fruiting from 85 to 108 days of age. It was noted in another study (**18**) that microsporogenesis precedes megasporogenesis by approximately 2 weeks and that the peak of syngamy occurs at approximately 65 days of age in the normally flowering gherkin. Because of artificial defloration in series 2, 3, 4, and fruit removal in series 3, it is seen that some of the series never attained certain of the developmental stages as defined. To facilitate comparison of data, however, the developmental stages have been set arbitrarily in all series as the time when they normally would have been at their peaks, if permitted, using series 1 as the standard.

The methods employed in obtaining data on growth and nutrient solution and the sampling and analytical procedures followed are in accordance with methods previously described (**17, 18**).

Results and discussion

Growth responses

Stem length.—The stem length of the main axis (fig. 1) increased slowly but uniformly in all series until the morphological appearance of floral structures. With the onset of budding just preceding 29 days of age, a stimulus in the rate of elongation was apparent. The total stem lengths attained at this time, however, or over the next 2 weeks, did not differ radically among the different series, and the deviations among series are easily within the limits of experimental error. In the flower development of the cucumber the onset of microsporogenesis normally precedes that of megasporogenesis; in another study (**18**) entailing an ontogenetical comparison between sexually intact and emasculated plants, such a stimulus to elongation accompanying the beginning of microsporogenesis at approximately 29 days of age was also noted. The succeeding acceleration in the rate of elongation concomitant with the beginning of megasporogenesis (occurring from 1–2 weeks later; approximately at 40 days of age) was observed in both studies. Data of the work reported earlier and the results of other investigators (**13, 22, 28, 29, 31, 37**) have suggested a correlation between autogenous fluctuations in relative growth rates (as stem elongation) and sporogenesis. The present study shows, however, that macroscopic staminate flowers were produced from 43 to 106 days and macroscopic pistillate flowers from 50 to 106 days (table 3). Presumably, then, microsporogenesis and megasporogenesis in some flowers was taking place, at least in series 1, over this entire period. The data of table 3 indicate that flowering was most intensive from 65 to 78 days. Acceleration in stem elongation is marked only after 71 days. The data (fig. 1, table 3) also suggest that, although the earlier less-pronounced points of inflection in the curves of stem elongation are coincidental with the onset of

microsporogenesis (29 days) and megasporogenesis (40 days), elongation and sporogenesis are not causally related, as all series showed a comparable increase in over-all length at least up to 65 days of age. In plants pollinated at known dates a stimulus to elongation also appeared concomitant with and subsequent to fertilization. This abrupt increase in the rate of elongation was the most pronounced of the entire growth cycle and has been recognized by other workers (**13, 29, 37**). In series 2 and 4, where fertilization was not permitted, however, the acceleration in the rate of elongation after 65 days of age equaled or exceeded the change in rate of the fruiting series 1 and 3, and at maturity stems of series 1 and 3 were consequently shorter than the nonfruiting plants (figs. 1, 3). A restriction in the vegetative growth of fruiting plants has been attributed to an inhibition owing to maximum fruit production (**27, 30, 37**). In the present work (and perhaps in others dealing with species of indeterminate flowering

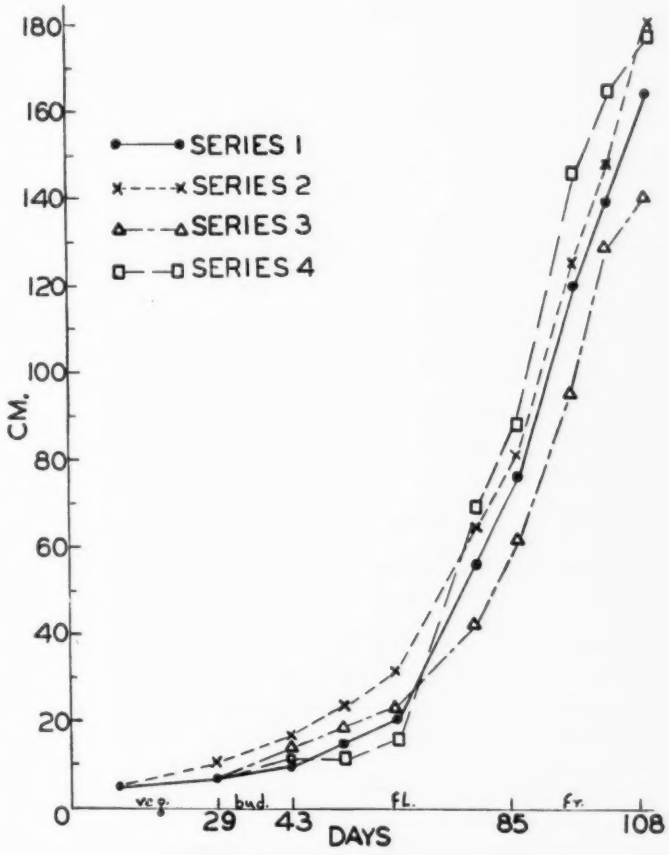

FIG. 1.—Stem elongation in centimeters

habit) the effect of the duration and intensity of flowering (microsporogenesis and megasporogenesis) upon the impetus in the stem elongation curve around 71 days should also be evaluated. The importance of the effects of other factors on the reproductive cycle, also, should not be overlooked. Particularly, it is suggested that a maximum production of growth substances initiated antecedent to the normal occurrence of these sexual phenomena and elicited by the formation of the flowering stimulus itself would account for the superior vegetative growth at these periods, even when normal flowering and fruiting were interfered with or restricted. Especially would this be true if the translocation to floral loci of hormonal substances produced in vegetative organs and normally channeled with available food to the developing flowers and fruit were curtailed by inactivation of pistillate flowers. The data of this report suggest that a complex similar to this might be operative, since series 2 and 4, in which female flowers were unpollinated or were eliminated at bud, showed the greatest total extension of the main axis. Also, pistillate flowers in the gherkin are generally produced upon lateral branches, and, if flowers are removed or inactivated, then the main axis of the plant should receive the full impetus of growth substances.

LEAF NUMBER and AREA.—Leaf inception and expansion (table 2) also showed periods of minima and maxima; relatively few leaves were developed in the period from 10 to 45 days, but the most rapid relative rate of enlargement occurred during this interval. Leaf numbers increased subsequent to first fertilization (after 50 days of age), but gains in foliar area on a leaf-number basis were relatively less. As was true for stem length, plants in which the female flowers were unpollinated or removed (series 2 and 4) showed the greatest leaf

TABLE 2

AVERAGE NUMBER OF LEAVES AND AVERAGE TOTAL LEAF AREA (SQ. CM.) PER PLANT

AGE IN DAYS	GROWTH PHASE	SERIES 1*		SERIES 2		SERIES 3		SERIES 4	
		No.	Area	No.	Area	No.	Area	No.	Area
10	Veg.	2	8	2	8	2	8	2	8
17	Veg.	4	42	4	45	4	40	3	35
24	Veg.	5	70	6	90	5	70	5	65
31	Bud.	6	120	7	147	6	120	5	98
38	Bud.	8	200	8	240	8	210	6	120
45	Flow.	10	360	12	520	10	400	7	285
52	Flow.	14	580	14	672	14	590	10	410
59	Flow.	18	1024	20	1200	19	1083	16	864
66	Flow.	23	1408	26	2020	24	1748	22	1650
73	Flow.	28	1700	34	2650	31	2410	29	2320
80	Flow.	35	2200	39	2775	32	2450	37	2800
87	Fruit.	37	2350	42	2940	34	2550	40	3050
94	Fruit.	40	2436	44	3740	41	2800	46	4230
101	Fruit.	42	2450	46	3910	43	3096	50	4700
108	Fruit.	42	2592	48	4850	45	4480	52	5504

* See table 1 for plan of experimentation.

number and area by the end of the experiment. The fruiting plants (series 1 and 3) produced fewer leaves and a significantly lower total leaf area. An apparent stimulus in leaf enlargement associated with the normal time of onset of micro- and megasporogenesis and a stimulus in the inception of new leaves following the usual time of fertilization were noted in *all* series. This precludes an assumption that such maxima in growth result solely from the influence of sexual processes and is further evidence that the enhanced leaf growth of series 2 and 4 was possibly due to optimum production of growth substances prior to and associated with the onset of these sexual phases of ontogeny. In unaltered plants growth agents would have been directed to the developing floral organs. LOEHWING (**24**), as well as others, has convincingly demonstrated that the loci of photoperiodic perception are confined to the leaves, and the work of AVERY and POTTORF (**3**) suggests a correlation between nitrogen supply, auxin content, and foliar area. Although specific growth substances may be instrumental in determining the developmental phases of ontogeny, the quantitative expression of these is, however, often a function of the food supply, as shown previously by differences in response of sexually modified plants at two levels of nitrogen supply (**18**). Also, differences in leaf number and area between normal fruiting controls and defruited plants (series 1, 3) exhibit the results of food monopolization by developing fruit and the retardation of vegetative growth even under favorable conditions of mineral nutrient supply (table 2).

FLORAL DEVELOPMENT.—Flower-bud formation was initiated simultaneously in all series (table 3). Quantitative differences in later initiation, however, were noted among the series. The lowest total number of flowers was produced by the series in which the female flowers were pollinated, with and without fruit (series 1 and 3); whereas the highest total number of flowers was produced by plants in which the pistillate flowers were inactivated by lack of pollination or removal (series 2 and 4). Both DEARBORN (**13**) and MCCOLLUM (**25**) have reported significant decreases in production of pistillate flowers by the cucumber in normal flowering and fruiting or defruited plants compared with deflorated or unpollinated plants. The same trend is evident in this research (table 3), although differences do not appear to be especially significant. The increased production of staminate blossoms by unpollinated plants agrees with the data of MCCOLLUM (**25**). Apparently staminate flower formation was affected differently, however, when plants were totally deflorated, as in DEARBORN's work (**13**), contrasted to the response to only female defloration in this study (series 4, table 3). The higher total number of flowers produced

TABLE 3

CUMULATIVE WEEKLY TOTAL FLOWER COUNTS PER PLANT (INTACT PLUS REMOVED) AS AVERAGE OF SERIES. SEX DETERMINED PRIOR TO REMOVAL

AGE IN DAYS	SERIES 1		SERIES 2		SERIES 3		SERIES 4	
	♂	♀	♂	♀	♂	♀	♂	♀
43	3	0	2	0	5	0	4	0
50	3	0	3	1	4	1	5	0
57	7	1	10	2	8	3	9	2
64	12	3	15	3	15	5	15	3
71	16	6	21	3	17	6	23	5
78	31	7	38	4	26	8	44	8
85	33	7	46	7	35	9	52	10
92	35	8	53	9	36	10	57	11
99	37	8	58	10	39	10	61	12
106	39	8	62	12	41	10	63	13

by the nonfruiting series 2 and 4 showed that the magnitude of flowering followed the same trend as vegetative stem and leaf responses (fig. 1 and table 2) of these series and indicates that, at least from a quantitative aspect, flowering is in reality a manifestation of growth.

Many workers have demonstrated that a higher content of auxin is found in reproductive structures than in vegetative structures and that auxin increases in the plant as a whole with the onset of the reproductive processes (**4, 19, 21, 32, 37**). WITTWER (**37**) concluded that the increased growth of plants subsequent to meiosis and fertilization was in part or wholly accounted for by the production of growth hormones within the reproductive organs. HATCHER (**19**) stated that in the ear development of rye there are two sites of auxin formation, anthers and carpels; but he also stated that it is untenable to associate maximum production of auxin in these structures with vegetative growth in cereals, as tillering precedes reduction division and "shooting" or maximum extension is completed prior to fertilization. LAIBACH and MEYER (**21**) found that corn seedlings 30 cm. high had no measurable auxin by the diffusion method, but with the production of male flowers there was a sudden appearance of growth substances, followed by a secondary maximum attending syngamy. AVERY ET AL. (**4**) reported production of auxin in greater amounts by reproductive shoots than by vegetative ones and recorded that the centers of hormone production were located in the terminal buds. In a later publication, AVERY and POTTORF (**3**) noted the presence of auxin in old leaves but to a lesser degree than in vigorously meristematic regions of the stem tip. AVERY and co-workers (**3, 5, 8, 9**) have studied also the chemical and physiological properties of auxin "precursor" and stated that, although auxin precursor is not auxin, it constitutes the major share of the potential auxin in the economy of the plant. In the present study, however, the superior vegetative and flowering responses of the nonfruiting series 2 and 4 serve to question the validity of interpreting the results on the basis of the occurrence of synapsis and syngamy alone when these were in part or wholly blocked. HATCHER'S (**19**) work with rye also questions this interpretation (**37**) in the development of cereals.

Instead, in light of the literature discussed above, it could be concluded that growth substances, or their precursors, produced in vegetative organs are translocated to and activated at formative reproductive centers. In the absence or restriction of the normal sequence of reproduction (series 2 and 4) and the failure of young ovules and ovaries to pre-empt the translocatable inorganic and organic materials available, the plant, under stimulus of quasicatalytic agents produced outside the reproductive structures, utilizes the organic reserves which normally would lead to the climax of reproduction; hence the enhanced vegetative and flowering vigor of series 2 and 4. This premise is further supported by the work of LOEHWING (**24**), who studied the photoperiodic response of the soy-bean plant by the slit-panel technique. His results showed that the flowering stimulus is obviously due to hormonic substances of foliar origin; by properly combining the effects of defloration and defoliation with photoperiodic illumination, he was able to demonstrate stimulus transfer and flower inception in normally vegetative regions. Similarly, MCCOLLUM (**25**) removed the leaves from the upper nodes bearing pis-

tillate flowers at the time of blossoming in the cucumber. The flowers were then pollinated, but fruit failed to develop, and he concluded that foliage near fertilized ovaries seemed to be essential for fruit development. DANIELSON (12) has also noted in the gherkin that vegetative response to daylength became especially minimal and maximal rates of water uptake per plant were associated with the usual time of occurrence of certain developmental stages despite sexual modification of plants and the failure of series 2, 3, and 4 to attain some of these stages. This has been previously reported by the author (17, 18) and by various workers

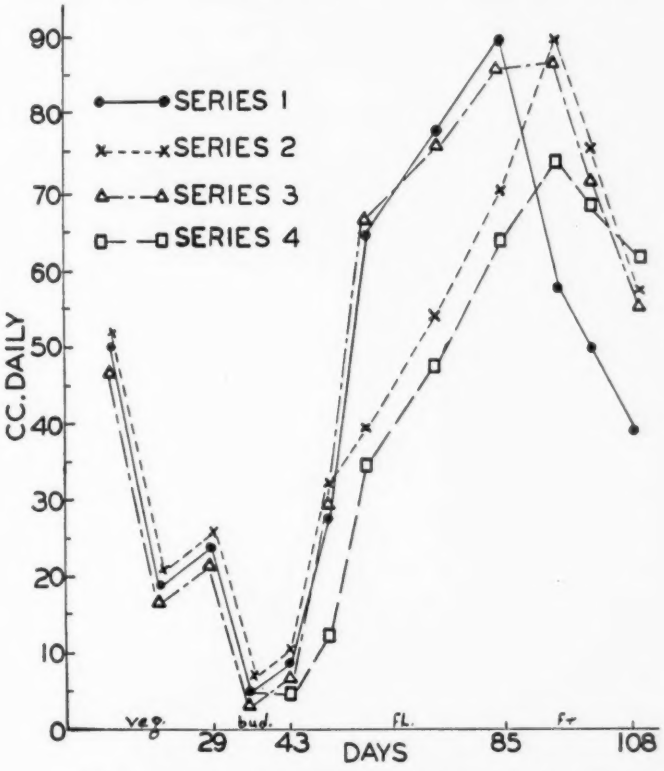

FIG. 2.—Average water consumption in cubic centimeters per day

evident with the onset of flowering and suggested that the photoperiodic reaction of the stem is physiologically related to the onset of flowering.

SUBSTRATE ALTERATIONS

A similar trend in absorption of water was observed for all series regardless of the defloration pattern (fig. 2). Periods of using other species (6, 14, 22, 31). The preflowering drop of moisture content has been suggested as a convenient index to the presence of floral primordia in many annuals (22). When water consumption was accurately measured, the sharpest decline in the absorption curve per plant also appeared at this time and was uniformly displayed by all series

(fig. 2). It is also interesting that, although the nonfruiting series 2 and 4 had a slightly higher percentage of tissue moisture at 65 days (table 5), they had absorbed a lower total amount of water (fig. 2) prior to this sampling. Lower water consumption was maintained throughout ontogeny by the nonfruiting series (2 and 4) compared with the fruiting plants (1 and 3), yet they exhibited greater metabolic efficiency in terms of accumulation of dry weight.

TABLE 4

PH VALUE OF NUTRIENT SOLUTION RESIDUES COMPARED WITH PH VALUE OF ORIGINAL SOLUTION

AGE IN DAYS	GROWTH PHASE	PH OF ORIGINAL SOLUTION	PH OF LEACHATES FROM			
			Series 1	Series 2	Series 3	Series 4
2	Veg.	6.5	6.8	6.8	6.8	6.8
8	Veg.	6.6	6.3	6.3	6.3	6.3
15	Veg.	6.6	6.0	6.0	6.0	6.0
22	Veg.	6.5	5.7	5.7	5.7	5.7
29	Bud.	6.5	5.6	5.6	5.6	5.6
36	Bud.	6.3	6.1	6.1	6.1	6.1
39	Bud.	6.2	5.8	5.8	5.8	5.8
43	Flow.	6.4	5.5	5.6	5.5	5.8
50	Flow.	6.4	5.4	5.7	6.0	5.8
57	Flow.	6.7	6.7	6.7	6.6	6.3
64	Flow.	6.6	5.7	6.4	5.9	6.4
71	Flow.	6.5	7.0	6.5	7.1	5.9
78	Fruit.	6.7	6.7	6.7	7.2	5.9
85	Fruit.	6.7	7.0	7.9	7.2	7.0
92	Fruit.	6.5	6.2	7.4	7.1	6.6
99	Fruit.	6.7	7.3	7.0	6.9	7.0
106	Fruit.	6.7	7.4	6.7	7.1	6.9

Alterations in pH value of the nutrient media in contact with the roots also seemed to be closely correlated with fluctuations in the rate of absorption of water and minerals and with certain stages of the life-cycle (table 4). During the first week of the experiment the pH values of the nutrient leachates were higher than that of the original solution. A gradual change in the opposite direction occurred as the growth rate accelerated at around 10 days of age. With the first inception and differentiation of flower buds, pH values of nutrient solution residues became temporarily static. Following budding and with blossom emergence at 43 days, however, hydrion concentration underwent a sharp increase, reaching a maximum the following week. A secondary minimum in pH change occurred at the time when flowers were first fertilized, but the sequential fruiting stage and the onset of maturity were associated with ascending pH values.

It has been previously noted that minima and maxima of pH values of the substrate appear to be associated with those phases of ontogeny characterized by low and high metabolic activity, respectively (**17**). It is further suggested that the increase in acidity accompanying accelerated growth results principally from the carbon dioxide evolved during increased root respiration and excretion, with selective absorption of cations predominating at these periods. Several workers have demonstrated that carbonic acid arising from roots normally is the most effective component that would affect substrate reaction (**11, 16, 34**), although VIRTANEN and LAINE (**36**) have reported excretion of organic and amino acids by roots of legumes. MILLER (**26**) stated that "carbon dioxide is an end product of respiration and since the intensity of respiration varies directly with the intensity of metabolic activity, it would be expected that the amount of carbon dioxide excreted by roots would vary during the growing season of the plant." This is corroborated by the work of KOSSOWITCH (**20**), who found that mustard plants in water cultures produced increasing amounts of carbon dioxide up to the time of blooming. The relation of

carbon dioxide evolution to maxima and minima in substrate pH is further confirmed by the work of BARKAKOV (7). He showed that carbon dioxide of soil was insignificant at the beginning of vegetative growth of several crop plants but increased rapidly and reached its maximum at the blooming period, then declined rapidly to the minimum at the ripening period. He also noted that, the greater the plant growth, the more carbon dioxide in the soil, and that the maximum of carbon dioxide evolution varied with the crop and arose mostly from root respiration. It is pointed out that microbiological evolution of carbon dioxide in soil studies as above should also be evaluated, but it is not considered to be an important factor in nutrient-solution cultures. Therefore, it is believed that trends in hydrion values of the media during ontogeny are an index to both the qualitative developmental stage and the metabolic activity of the plant.

Weekly analysis of solution residues

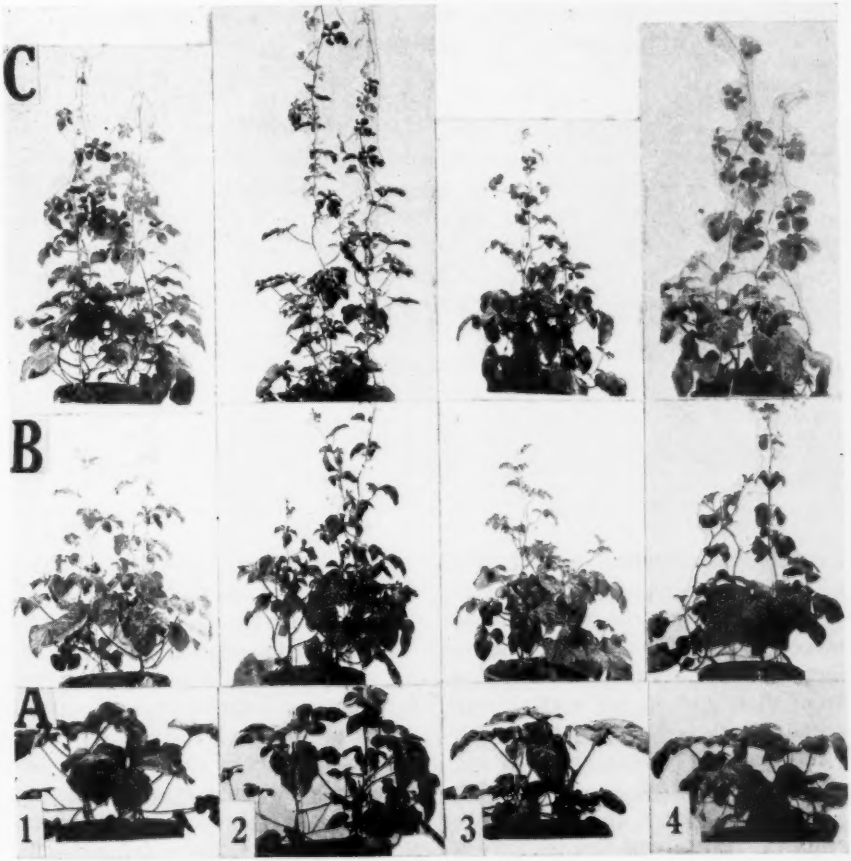

FIG. 3.—Representative plants photographed at three stages. *A*, vegetative stage; *B*, flowering stage; *C*, fruiting stage. *Left to right*, series 1, series 2, series 3, series 4.

provided an index to the nutritional status of the various series throughout ontogeny. Minima and maxima of salt absorption rather closely paralleled those for water consumption and pH values. The general pattern of absorption of inorganic nutrients from the substrate was essentially that previously described for the gherkin (**17**) and for herbaceous annuals (**22**). Therefore, formal data on this aspect of the experiment have been omitted from this report. Existing literature seems to justify the conclusion that changes in absorption of inorganic nutrients and the altered internal distribution of these and organic materials are associated with and possibly determined by the onset of reproductive processes (**1, 2, 10, 15, 22, 28, 29, 35, 37**). The data of this study showed that fluctuations in substrate alterations also occurred in all series when brought to the flowering stage, irrespective of subsequent sexual modification of plants, and suggest the influence of preformed hormonic agents in the regulation of these processes as well as of other more easily recognized growth changes.

Chemical composition

The plants with female flowers unpollinated or removed (series 2 and 4) consistently elaborated a greater fresh and dry weight than did the pollinated series (1 and 3) with and without fruit at both periods of sampling (table 5). From 65 to 108 days the most marked increase in fresh weight occurred in the nonfruiting series, and this difference from fruiting plants was even more accentuated in terms of dry weight (**25, 37**). Fresh weights of roots of series 2 and 4 were proportionately higher than those of series 1 and 3 at 65 days, but at 108 days fresh weights of roots did not differ significantly among series except in the case of series 3 (table 5). Analyses at 108 days of age showed that top/root ratios

TABLE 5

FRESH WEIGHT, DRY WEIGHT, ASH WEIGHT (GM. PER PLANT), AND PERCENTAGE DRY WEIGHT AT TWO PERIODS OF SAMPLING

Fraction	Series 1			Series 2			Series 3			Series 4		
	Root	Shoot	Top/Root	Root	Shoot	Top/Root	Root	Shoot	Top/Root	Root	Shoot	Top/Root
	At 65 days of age (flowering)											
Fresh wt.	4.8	22.7	4.7	13.7	56.6	4.1	5.6	40.6	7.3	9.4	55.3	5.8
Dry wt.	0.6	3.0	5.0	1.6	12.1	7.6	0.9	7.9	9.2	1.3	10.4	8.2
% Dry wt.	12.5	13.2	.....	12.0	21.3	.....	15.5	19.6	.....	13.5	18.9	.....
Ash wt.	0.2	0.5	.....	0.3	2.1	.....	0.2	0.8	.....	0.2	1.6	.....
	At 108 days of age (mature fruit)											
Fresh wt.	10.0	60.1	6.0	10.4	79.3	7.6	7.0	59.8	8.5	11.5	102.6	8.9
Dry wt.	1.1	9.1	8.2	1.4	13.9	9.8	1.0	9.3	9.6	1.3	20.5	15.8
% Dry wt.	11.1	15.1	.....	13.7	17.5	.....	13.8	15.6	.....	11.3	20.0	.....
Ash wt.	0.2	2.3	.....	0.3	2.3	.....	0.1	1.4	.....	0.2	3.2	.....

were uniform in all series when expressed as fresh weight, but dry-weight ratios showed a much greater gain in series 4 and emphasized the rapid and enhanced shoot growth of this series (table 5). ARNON and HOAGLAND (2) have noted that deflowered tomato plants produced a greater fresh weight when growing on a complete solution than did fruiting plants under the same conditions.

lower absolute and relative amounts of total carbohydrates than series 2 and 4, and this relationship still existed at the final sampling (table 6). Both soluble sugars and hydrolyzable polysaccharides increased in actual amounts in all series with age, but, when expressed as percentage of the dry weight produced, both fractions declined in the series with intact male flowers (series 1 and 4).

TABLE 6

CARBOHYDRATE AND NITROGEN CONTENT (MG. PER PLANT) AND PERCENTAGE DRY WEIGHT AT TWO PERIODS OF SAMPLING

Fraction	Series 1		Series 2		Series 3		Series 4	
	Wt.	%	Wt.	%	Wt.	%	Wt.	%
	At 65 days of age (flowering)							
Soluble sugars	99.6	2.7	452.4	2.7	221.8	2.5	395.7	3.4
Polysaccharides	662.0	18.3	3233.7	19.2	1570.2	17.8	2284.5	19.5
Total carbohydrates	761.6	21.0	3686.1	21.9	1792.0	20.3	2680.2	22.9
Soluble nitrogen	21.3	0.6	113.0	0.7	62.7	0.7	64.6	0.6
Insoluble nitrogen	13.7	0.4	41.3	0.2	19.4	0.2	34.5	0.3
Total nitrogen	35.0	1.0	154.3	0.9	82.1	0.9	99.1	0.9
	At 108 days of age (mature fruit)							
Soluble sugars	249.6	2.5	494.6	3.2	386.1	3.7	559.1	2.6
Polysaccharides	1002.7	9.8	4350.6	28.5	1513.1	14.7	3726.9	17.1
Total carbohydrates	1252.3	12.3	4845.2	31.7	1899.2	18.4	4286.0	19.7
Soluble nitrogen	72.3	0.7	58.1	0.4	39.1	0.4	93.8	0.4
Insoluble nitrogen	115.0	1.1	67.3	0.4	103.0	1.0	105.8	0.5
Total nitrogen	187.3	1.8	125.4	0.8	142.1	1.4	199.6	0.9

Accumulation of ash constituents followed the trends in fresh and dry weights at the two dates of analyses. The shoots of the gherkin contain the major fraction of ash in the entire plant (table 5). The high ash weight of series 4 is in agreement with the work of LOEHWING (23) and STANFIELD (33), who recorded data showing that staminate plants invariably accumulate more ash constituents than do comparable pistillate plants.

The early analysis disclosed that the fruiting series 1 and 3 produced both

LOEHWING (23) and STANFIELD (33) noted that male plants of the dioecious species of hemp, spinach, and *Lychnis* were characterized by a higher carbohydrate content than female plants, while ARNON and HOAGLAND (2) reported that deflowered tomato plants accumulated seven times the starch content of normal fruiting plants. Their findings are in harmony with the data for the artificially induced male series of this research (series 4, table 6).

At 65 days of age the pollinated plants,

with and without fruit (series 1, 3), showed slightly higher percentages of total nitrogen on the dry-weight basis (if computations are made to the second decimal place) than the nonfruiting series; by 108 days this difference between fruiting and nonfruiting plants was highly significant (table 6). DEARBORN (13) has noted comparable results for sexually modified cucumber plants when the total nitrogen content was expressed as percentage of total dry weight. The nitrogen composition of the plants at the first sampling indicated that the major fraction of the total nitrogen was in the soluble form in plants of all series (table 5); at 108 days, however, the major part of the total nitrogen content had shifted to the insoluble form.

Summary

1. The physiological responses and growth of the normally monoecious gherkin, *Cucumis anguria*, were studied under varying degrees of artificially imposed dioecism and compared with those of normal plants throughout ontogeny, grown under similar environmental conditions.

2. Minimal and maximal periods in the rate of vegetative growth were noted. Despite sexual modifications, all series followed closely the basic trends described, differing only in the quantitative aspect. The induced dioecious series (2 and 4), in which pistillate flowers were unpollinated or removed consistently, excelled in magnitude of vegetative stem and leaf, and flower, production. The postulated role of growth substances as a possible factor in accounting for the ontogenetic fluctuations is discussed.

3. Alterations in the rate of absorption of water and nutrients and changes in the reaction of the substrate seemed to be closely correlated with certain stages of the life cycle. A preflowering drop in the rate of water and mineral uptake and pH changes of the nutrient cultures are suggested as indexes to the presence of flower primordia in the gherkin, often before they are macroscopically visible. The consistency among series in displaying these substrate alterations suggests also the influence of preformed hormonic agents in the regulation of these processes.

4. At the final sampling the fruiting plants had the lowest total fresh, dry, and ash weights; and total carbohydrates; but the highest relative amounts of total nitrogen.

5. It is concluded that, if normal flowering and fruiting are interfered with, metabolic derangements and alterations result. A possible explanation for the superiority of nonfruiting plants in most growth increments might be due to utilization of accumulated organic reserves, under the stimulus, and perhaps impaired translocation, of growth substances produced outside the reproductive structures. These normally would lead to flowering and fruiting. It is also suggested, as has been done by others, that the apparent stimulation concurrent with the time of the reproductive processes may stem not directly as the result of these phenomena but indirectly in association with maximum production of growth substances at these periods affecting the entire plant.

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COMPLEX GROWTH REQUIREMENT OF AN ARGININE-LESS MUTANT OF NEUROSPORA[1]

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Introduction

From studies of biochemical mutant strains of *Neurospora*, and from other investigations as well, there has accumulated a mass of evidence which indicates that particular genes act to control specific biochemical reactions (1). This would seem to imply but does not in itself prove that the primary function of a gene is to determine the specificity of an enzyme. For critical discussion of the experimental basis for this idea see BEADLE (3). Relative to the question of whether genes and enzymes have a one-to-one relationship, a consideration of some importance has been that thus far among biochemical mutants of *Neurospora* not many single-gene mutants with double growth-factor requirements have appeared, and only a few have been studied extensively (4, 9). The present paper reports preliminary work on another mutant which may be of this type in that it has the following attributes: (*a*) it is unable to grow on minimal medium; (*b*) this failure in metabolism is apparently inherited as a single-gene difference from the wild type; (*c*) no single substance which has been tested will satisfy the growth requirement of this mutant strain, but combinations of arginine or citrulline with certain purines or pyrimidines elicit striking growth responses.

Analysis of this mutant strain has not proceeded sufficiently far to permit interpretation of its characteristics in terms of theories of gene action, but the strain presents a number of points of rather immediate interest. First, the mutant character in this strain represents a genetic block intervening between citrulline and its precursor substance ornithine that is biochemically and genetically different from two others previously described (8). Second, the mutant strain is noteworthy for the number of possible substituents which are able, at least in part, to fulfil that portion of the growth-factor requirement which is not satisfied by arginine. Finally, evidence so far uncovered is consistent with the supposition that this is an authentic instance of a single-gene mutant with a double growth-factor requirement.

Material and methods

Details as to general methods of culturing *Neurospora* are readily available elsewhere (6). Unless otherwise designated, growth experiments reported in this paper were carried out in 125-ml. Erlenmeyer flasks, each containing 20

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ml. of liquid culture medium. Incubation was at 25° C. Since at best the strain of *Neurospora* with which this paper is primarily concerned is relatively slow-growing, the incubation period was lengthened from the usual 72 hours to periods up to 120 hours. Growth was measured by determining the dry weight of mycelia produced after given lengths of time.

Techniques in making genetic crosses and methods of genetic analysis in *Neurospora* are summarized in a review paper by BEADLE (**2**).

MUTANT STRAINS.—Strain 30820, the chief object of description in this paper, was obtained for investigation by the author from Dr. G. W. BEADLE at Stanford University in 1946. Strains 29097, 30300, and 33442, also mentioned briefly, were obtained from Dr. BEADLE and Dr. E. L. TATUM, also at Stanford, at an earlier date. Since first received for study, all these strains have been outcrossed one or more times to wild-type *Neurospora*, with the mutant genes which primarily differentiated the original strains being recovered among the progeny of such crosses. This means that the strains as presently being investigated may not behave in identical fashion with the originals, since the mutant genes are now undoubtedly operating against somewhat different genic backgrounds. There is no evidence, however, that in the process of outcrossing and recovery the mutants have become associated with outstanding modifiers which drastically alter the qualitative characteristics of the strains as determined by growth responses.

Results

GROWTH REQUIREMENTS OF STRAIN 30820.—When strain 30820 is inoculated onto minimal medium in the liquid form, it is characteristic that no growth is detectable for 6–8 days. After such a period of time, slow growth may be initiated. In experiments so far, the time for adaptation has not been accurately predictable, in that, in a single experiment among replicate treatments given identical inocula, both the initiation and the rate of growth after about a week's time have been irregular for this mutant. Such behavior may be compared with that of the wild-type controls used in these investigations, where growth for 72 hours on minimal medium yields an average of 63 mg. in dry weight.

Strain 30820 initiates growth almost immediately if the minimal medium is supplemented with yeast or malt extract. There is no response, however, to a mixture of synthetic vitamins including thiamin, riboflavin, pyridoxin, pantothenic acid, *p*-aminobenzoic acid, nicotinic acid, choline, and inositol. On the other hand, tests with hydrolyzed casein show slight but definite growth activity. Of individual amino acids the following do not promote growth: *L*-lysine, *DL*-serine, *DL*-threonine, *L*-histidine, *DL*-valine, *DL*-leucine, *DL*-isoleucine, *DL*-norleucine, *L*-cysteine, *DL*-methionine, *DL*-α-amino caproic acid, *DL*-phenyl alanine, *L*-hydroxy proline, *L*-proline, *L*-cystine, *L*-aspartic acid, β-alanine, *DL*-glutamic acid, *L*-tryptophan, *DL*-α-amino adipic acid, carbamyl-*L*-glutamic acid, and glycine. Addition of *L*-arginine does elicit some growth response, however, and the mutant grows as well or better on comparable concentrations of *DL*-citrulline. So far, growth on ornithine has not occurred under any conditions tried. These facts would seem to indicate that in this strain the biosynthesis of arginine fails because of a genetic inability to convert ornithine into citrulline (**8**). But, although the growth response of strain 30820 to arginine or to citrulline is defi-

nite, it is a response which quantitatively is very weak when compared with results of supplying arginine or citrulline to other mutant strains of *Neurospora* unable to convert ornithine into citrulline. For example, growth of strain 33442 is 50 mg. and that of strain 30300 is 48.5 mg. in dry weight after 3 days, while 30820 produces only 19 mg. after 5 days on 50 micromoles of arginine. Both strains 33442 and 30300 can be brought to the growth rate of wild-type *Neurospora* if supplied with a sufficient quantity of arginine or citrulline. This has been

TABLE 1

EFFECT OF ADENINE ON THREE DIFFERENT MUTANT STRAINS UNABLE TO SYNTHESIZE ARGININE. VALUES REPRESENT DRY WEIGHTS IN MG. OF MYCELIA AFTER INCUBATION PERIOD OF 80 HOURS

SUPPLEMENT TO MINIMAL MEDIUM	STRAINS		
	30820	30300	33442
None	0.0	0.0	4.8
1 mg. adenine-H_2SO_4	0.8	0.0	5.1
5 mg. arginine-HCl	8.2	46.2	52.4
1 mg. adenine-H_2SO_4+5 mg. arginine-HCl	29.6	46.0	51.8

far from true for strain 30820. In other words, citrulline or arginine in itself does not provide a sufficient answer to the growth requirement of this latter mutant strain.

In view of the possibility that mutation of wild type had given rise in mutant 30820 to a strain with a double or multiple growth-factor requirement, arginine was tested in combination with each of the amino acids listed above which had proved to be inactive alone. No positive responses were noted. Neither was the combination arginine plus vitamin mixture more effective than arginine controls. It is demonstrable, however, that a combination of arginine or citrulline plus adenine gives striking growth increases when compared with controls where arginine or citrulline is the sole supplement to the minimal medium. Adenine as a sole supplement to minimal medium permits almost no growth of the mutant strain. The combination arginine plus adenine is no more effective for the growth of strains 30300 and 33442 than is the same concentration of arginine alone (table 1).

GENETIC PROPERTIES OF STRAIN 30820.—Strain 30820 has been crossed to strain 30300 and also to strain 33442. Analysis of the results of these crosses makes it clear that in each of the strains the inability to grow on minimal medium is conditioned by a different single gene.

With the possibility of a double growth requirement in strain 30820, it becomes of more than ordinary interest to determine whether the mutant characteristics of this strain are due to the effect of a single gene or of more than one gene. To get at an answer to this question, mutant strain 30820 was crossed with wild-type strains of opposite mating type. Ascospores obtained from the crosses were isolated at random. Each culture established by germination of such a spore was then tested for its ability to grow on minimal medium. In each of three separate experiments in which ascospores were isolated at random, results obtained were in reasonable agreement with the 1:1 ratio of growers to nongrowers which would be expected if inability to grow on minimal medium were inherited as a single gene. These experiments are summarized in table 2.

In addition, each of the segregants which failed to grow on minimal medium was subjected to test-tube experiments to determine its ability to grow on arginine alone, on adenine alone, and on the com-

bination of arginine plus adenine. To make observations relative to growth more definitive, these test-tube experiments were carried out in 18- × 150-mm. culture tubes, each containing 5 ml. of medium, instead of in the 10- × 75-mm. size often used in genetic analyses involving biochemical mutants of *Neurospora*. In all but sixteen cases it was visibly obvious after 3 days that the combination treatment gave by far the best growth response. The sixteen questionable cultures were subjected to retest in Erlenmeyer flasks, in the same manner as original strain 30820 was tested in the experiment shown in table 1. In each instance the mutant isolates from among the progeny showed a growth response essentially that of the parent mutant culture.

Another form of genetic approach to the problem of whether the mutant characteristics of strain 30820 are due to the effects of a single gene was employed in a limited number of instances where ascospores were dissected in order out of asci formed after crosses between 30820 and the wild type. Results of tests of cultures so derived were consistent with expectation on the basis of a single-gene segregation within the ascus. Out of thirty asci dissected in order, the mutant character segregated at the second division of meiosis in ten instances, indicating the mutant gene to be about sixteen and one-half map units distant from the centromere. The linkage relations of the mutant gene in strain 30820 have not yet been established.

GROWTH RESPONSES OF STRAIN 30820 TO PURINE AND PYRIMIDINE COMPOUNDS.—Besides adenine, certain other purine and certain pyrimidine compounds stimulate growth of strain 30820 if supplied in combination with arginine or citrulline. None of these compounds is active alone or in combination with ornithine. Effects of amino acid and purine or pyrimidine combinations on growth of strain 30820 are presented in table 3. Since optimum concentrations for maximal growth of strain 30820 have not been worked out for all the combinations, the comparative effectiveness of these combinations is not necessarily revealed by the values reported.

GROWTH RESPONSE OF STRAIN 30820 TO UREA.—It is noteworthy that so many purine and pyrimidine compounds are active in promoting growth of strain 30820 far beyond what is attainable on arginine or citrulline alone. Among previously described mutants of *Neurospora*, none concerned with nucleic acid metabolism (7) has had growth requirements that could be satisfied by so great a variety of substituent compounds. This might suggest that in the case of strain 30820 it is not the purine or pyrimidine compounds themselves but some common derivative of them that is the active metabolite. Urea, a compound

TABLE 2

SEGREGATION OF INABILITY TO GROW ON MINIMAL MEDIUM AS FOUND AMONG CULTURES DERIVED FROM SINGLE ASCOSPORES ISOLATED AT RANDOM FROM CROSSES OF WILD-TYPE NEUROSPORA × MUTANT STRAIN 30820

Cross number	% Germination among isolates	Cultures growing on minimal medium	Cultures not growing on minimal medium	Chi-square values for goodness of fit to 1:1 ratio	*P* value of larger chi-square value exceeds
I	93	89	79	0.60	0.30
II	87	139	148	0.28	.50
III	80	202	176	1.79	0.10
Total		430	403	0.88	0.30

thought to be one end product of purine and pyrimidine metabolism in certain organisms (5), has been found to have a stimulatory effect on mutant 30820 (table 4). This substance shows growth activity only when present in the culture medium along with arginine or citrulline. Urea alone, or urea plus guanylic acid or other purine or pyrimidine compounds that have been tested, is only slightly if at all effective in promoting growth of the mutant. It is not likely, however, that the active purine or pyrimidine compounds listed in table 3 function merely to supply urea to the mutant strain; compounds like guanylic acid are relatively far more effective in stimulating growth than is urea itself. Table 4 shows, for example, that if each supplement is in combination with the same concentration of arginine, 3 micromoles of guanylic acid give rise to more growth over a 108-hour period than do 500 micromoles of urea. And in experiments so far the greatest stimulation by urea has occurred in concentrations of around 500 micromoles in 20 ml. of culture medium. All the urea used in these experiments was sterilized by filtration, and experiments with buffered solutions indicate that the urea effect is not a pH effect. Neither can the effect of urea be simulated by increasing ammonium nitrogen in the medium.

TABLE 3

GROWTH RESPONSE OF STRAIN 30820 TO COMBINATIONS OF AMINO ACIDS AND PURINES OR PYRIMIDINES, AS MG. OF DRY WEIGHT OF MYCELIUM; EACH VALUE THE MEAN FOR TREATMENT RUN IN DUPLICATE; INCUBATION PERIOD, 86 HOURS

PURINE OR PYRIMIDINE SUPPLEMENT (1 MG./CULTURE FLASK) TO MINIMAL MEDIUM	AMINO ACID SUPPLEMENT ($M/400$) TO MINIMAL MEDIUM			
	L-arginine-HCl	*DL*-citrulline	*DL*-ornithine	None
None	8.0	10.3	0.0	0.0
Adenine-H_2SO_4*	36.0	39.4	.0	.2
Guanine-H_2SO_4	25.0	27.2	.0	.0
Uridine	27.2	24.3	.0	.0
Guanylic acid*	33.8	37.9	.0	.0
Xanthine	11.0	21.0	.0	.0
Hypoxanthine	13.3	26.5	.0	.0
Cytidylic acid*	21.4	21.7	.0	.0
Orotic acid	6.3	6.0	.0	.0
Uric acid	0.9	7.2	.0	.0
Thymine	10.2	9.3	0.0	0.0

* These compounds in this experiment were sterilized by filtration instead of usual autoclavation to test whether stimulatory effects previously obtained might be due to effects of reactions going on in autoclave instead of being carried out by mold. In same series as above, two adenine replicates were also sterilized by autoclavation for each of combinations. Average value with arginine was 36.4 and with citrulline was 40.0 mg.

TABLE 4

EFFECT OF UREA ON GROWTH OF STRAIN 30820; PERIOD OF INCUBATION, 108 HOURS

Supplement* to minimal medium	Dry weight† in mg.
None	0.0
Arginine	18.5
Urea	0.3
Guanylic acid	2.3
Arginine, urea	30.3
Arginine, guanylic acid	53.6
Urea, guanylic acid	1.9
Arginine, urea, guanylic acid	56.4

* When present, arginine was added as 26 micromoles per culture flask; guanylic acid as 3 micromoles per culture flask; and urea as 500 micromoles per culture flask.

† Each value is mean value for three identical treatments.

GROWTH REQUIREMENTS OF DOUBLE-MUTANT STRAIN 30820-29997.—BONNER (4) has already described an instance of a single-gene mutant strain of *Neurospora* having a double growth-factor requirement. In this case it appears that mutation of a gene has resulted not only in a defective mechanism for synthesizing one essential metabolite but also has the effect of accumulating behind the genetic block a compound which inhibits the biosynthesis of a second growth substance. It is conceivable that this kind of

interpretation might ultimately apply to the growth requirements of strain 30820.

Since strain 30820 represents a block in the conversion of ornithine to citrulline (**8**), there is at least the possibility that behind this genetic block a substance accumulates which acts in an inhibition that may be relieved by such compounds as adenine. To begin to test this possibility, a double-mutant strain was obtained from a cross between 30820 and strain 29997, which has a growth-factor requirement that can be satisfied by arginine, citrulline, or ornithine. In such a double-mutant strain, citrulline cannot be formed from ornithine, and presumably neither can ornithine itself be formed. Therefore, in this double mutant there should be no possibility of an accumulation of immediate precursors of citrulline which might act as metabolite inhibitors.

Table 5 shows the analysis of one of the asci dissected in order after the cross 30820a × 29997A. From the four spore pairs represented in this ascus, strains were derived which (*a*) had the single-mutant gene from strain 30820; (*b*) had the single-mutant gene from strain 29997; (*c*) had the wild-type alleles of the mutant genes from strains 29997 and 30820; and (*d*) were double-mutant strains. These derived strains were identified and classified on the basis of their biochemical characteristics and evidence from artificially produced heterocaryons. The double-mutant strains were proved to be double by outcrossing them to wild type and recovering both single-mutant genes among the progeny.

TABLE 5

ANALYSIS* OF ASCUS F, DISSECTED IN ORDER AFTER CROSS 30820a × 29997A. DRY WEIGHTS WERE TAKEN AFTER 72 HOURS

Supplement	Spores 1 and 2 (wild type)	Spores 3 and 4 (29997)	Spores 5 and 6 (30820)	Spores 7 and 8 (30820-29997)
None (minimal medium)	44.0	0.0	0.0	0.0
Arginine	51.2	44.9	2.5	0.9
Citrulline	47.3	51.5	1.6	4.3
Ornithine	39.0	34.0	0.0	0.0
Adenine	38.3	0.0	0.0	0.0
Arginine plus adenine	37.3	37.0	26.4	23.6
Ornithine plus adenine	37.0	18.0	0.2	0.0

* Biochemical tests made in 125-ml. Erlenmeyer flasks, each containing 20 ml. of liquid culture medium. Adenine added as 1 mg/20 ml, and each amino acid when present was in concentration of 50 micromoles per flask.

Discussion

Double-mutant strain 30820-29997 resembles strain 30820 in requiring both arginine or citrulline plus adenine for growth. This indicates that the adenine portion of the double requirement is not due to inhibition effects of some ornithine derivative which accumulates when ornithine cannot be converted into citrulline. On the other hand, the inability of the mutant to convert ornithine into citrulline may itself be the secondary effect of a genetic block which primarily concerns some other biochemical reaction.

Two facts emphasize that the basic growth requirements of strain 30820 are still imperfectly understood. First, in experiments to date even the most effective concentrations of arginine with purine or pyrimidine compounds have failed to bring the growth rate of the mutant up to the rate of wild-type growth. Second, the relatively large number of purine or pyrimidine compounds which stimulate growth suggests that a derivative of them rather than these compounds themselves may have the true growth-factor activity. The possibility also remains that a single com-

pound having to do with both arginine and purine metabolism will be found to satisfy the growth requirement of the mutant strain.

Summary

1. *Neurospora* biochemical mutant strain 30820 represents a third different instance of a genetic block in the conversion of ornithine to citrulline.

2. The inability of strain 30820 to grow on minimal medium is apparently inherited as a single-gene difference from wild type.

3. A supplement of arginine or citrulline to minimal medium does not in itself suffice to allow for good growth of strain 30820, but, if in addition to one of these compounds adenine is present, striking growth responses obtain.

4. Other compounds which give more or less the same effect as adenine include guanine, guanylic acid, uridine, and perhaps xanthine and hypoxanthine.

5. Urea in the presence of arginine or citrulline also substantially stimulates the growth of this mutant strain. Urea, however, is far less active than guanylic acid or certain other purine or pyrimidine compounds.

6. Essentially the same growth requirements as for strain 30820 are shown by double-mutant strain 30820-29097. In this strain the formation of ornithine as well as the conversion of ornithine to citrulline is genetically blocked.

7. Some discussion is made of difficulties which presently stand in the way of accepting and interpreting strain 30820 as a straightforward instance of a single-gene mutant with a double growth-factor requirement.

Several of the compounds used in these experiments were obtained through the generous courtesy of other investigators. Thanks are due to Dr. P. P. COHEN, of the University of Wisconsin, for the carbamyl-*L*-glutamic acid; to Dr. H. K. MITCHELL, of the California Institute of Technology, for the orotic acid; and to Dr. P. LOWY, also of the Institute, for a sample of α-amino adipic acid.

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RESPONSE OF FLOWERS OF BLACK CORINTH AND FRUIT OF *THOMPSON SEEDLESS GRAPES TO APPLICATIONS* OF PLANT GROWTH-REGULATORS

ROBERT J. WEAVER AND W. O. WILLIAMS

Introduction

Plant growth-regulators have been applied to the flowers or fruits of a great variety of plants (**1–6, 8–10**). Induction of fruit-set, seedlessness, hastening of maturity, prevention of preharvest fruit-drop, and an increase in fruit size are some of the reported responses.

Few studies of this type have been made with grapes. GARDNER and MARTH (**4**) did not obtain parthenocarpy by treating flowers of the Brighton grape with naphthaleneacetic acid. PENTZER (**9**) found that naphthaleneacetic acid applied to several varieties of grapes prior to picking failed to reduce the amount of "shattering." The purpose of the preliminary experiments performed in 1949 and reported here was to observe the response of flowers of Black Corinth and fruit of Thompson Seedless grapes to applications of various growth-regulators. These grapes are seedless varieties of *Vitis vinifera*.

Material and methods

The irrigated vineyard used was located about 1 mile west of Davis, California. Rooted cuttings of Black Corinth and Thompson Seedless vines were planted in 1933. The vines were cane pruned, a practice necessary for obtaining high yields from these varieties. Each vine of Thompson Seedless had four canes, six to ten shoots per cane, and about forty clusters of fruit, although there was much variation.

The mean monthly temperatures during the experimental period, May to September, inclusive, were 64°.7, 72°.2, 73°.8, 71°.1, and 71°.5, respectively.

Ten plant growth-regulators (table 1) were obtained from commercial sources. Oil emulsions were prepared by dissolving separately 1 gm. of each compound in 5 ml. of an oil-base carrier,[1] and then diluting to 1000 ml. with distilled water, except that 10 ml. of carrier were utilized to dissolve the beta-naphthoxyacetic acid. These stock emulsions were further diluted with water to make the desired concentrations. Additional carrier was added to the diluted emulsions to obtain a uniform concentration of 0.2% by volume. Aqueous solutions were prepared also by adding sufficient ammonia to dissolve the compounds and by adding Oronite, 0.1% by weight, as a wetting agent. At the concentrations used, preparations of Oronite or of carrier alone caused no injury.

Clusters of flowers or fruit were immersed momentarily in a formulation of one of the growth-regulators. Each treatment with Black Corinth consisted of six clusters. Each vine of Thompson Seedless was divided into two approximately equal parts. All clusters on one part were treated, and the remainder served as untreated controls.

Experimentation and results

BLACK CORINTH

The first treatments, utilizing all the formulations indicated in table 1, as well

[1] Plant Hormone Carrier W.E. 1709 supplied by Sherwin-Williams Company, Chicago, Illinois.

TABLE 1

DEGREE OF INJURY* RESULTING FROM GROWTH-REGULATORS APPLIED IN OIL EMULSION TO CLUSTERS OF BLACK CORINTH AND TO THOMPSON SEEDLESS GRAPES

Growth-regulator	Black Corinth			Thompson Seedless			
	Concentration of growth-regulator (p.p.m.)	Time of application		Concentration of growth-regulator (p.p.m.)	Time of application		
		May 11	May 20		June 7	July 7	July 28
Beta-naphthoxyacetic acid	200	++	++	500	+	†	o
	20	+	o	200	o	†	o
	2	o	o	20	o	o	o
Alpha-naphthaleneacetic acid	200	+++	+++	200	++	o	o
	20	+++	o	20	o	o	o
	2	++	o	.....	.....	.....	.....
Gamma-(indole-3)-*n*-butyric acid	1000	+++	+++	500	+	o	o
	200	++	o	50	o	o	o
	20	o	o	.....	.....	.....	.....
Beta-naphthoxypropionic acid	200	+++	+	1000	+++	++	+
	20	++	o	500	++	+	+
	2	o	o	200	+	o	o
	.....	.....	.....	20	o	o	o
	.....	.....	.....	2	o	o	o
2,4,5-Trichlorophenoxyacetic acid	50	.....	+++	50	+++	+++	+
	5	+++	+++	5	++	+	o
	0.5	++	o	0.5	o	o	o
4-Chlorophenoxyacetic acid	50	.....	+	200	+	+++	o
	5	o	o	20	o	+	o
	0.5	o	o	2	o	o	o
2,4-Dichlorophenoxyacetic acid	200	.....	+++	.....	.....	.....	.....
	20	+++	+++	.....	.....	.....	.....
	2	+++	++	.....	.....	.....	.....
	0.2	+	o	.....	.....	.....	.....
2-Chlorophenoxyacetic acid	50	.....	o	.....	.....	.....	.....
	5	o	o	.....	.....	.....	.....
	0.5	o	o	.....	.....	.....	.....
Alpha-(2-chlorophenoxy)propionic acid	50	.....	+++	.....	.....	.....	.....
	5	+	++	.....	.....	.....	.....
	0.5	o	o	.....	.....	.....	.....
Indole-3-acetic acid	200	.....	++	.....	.....	.....	.....
	20	+	+	.....	.....	.....	.....
	2	o	o	.....	.....	.....	.....

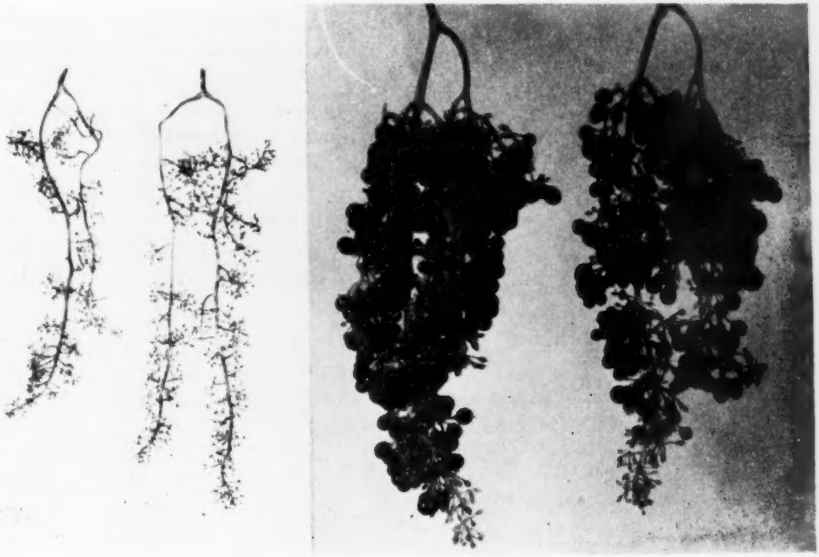
* o = No injury.
\+ = Slight injury. Usually a slight curvature of rachis occurred. Cluster recovered and developed normally, except that some berries often failed to enlarge.
++ = Medium injury. Many berries failed to enlarge. Rachis usually enlarged, split, and callused. Tip of cluster was sometimes dead.
+++ = Severe injury. Cluster dead and dry, or if alive, less than 5% of berries had enlarged, and rachis was twisted and split. Berries of treated Thompson Seedless were dehydrated.

† Crop lost.

as aqueous solutions of beta-naphthoxyacetic acid, alpha-naphthaleneacetic acid, and gamma-(indole-3)-*n*-butyric acid at the same concentrations, were made on May 11, when about 10% of the flowers on the clusters were in bloom. At the later treatments on May 20 a high percentage of the flowers was in bloom. At that time the trunks of four vines were girdled, but their clusters were not treated.

RESULTS OF LATER TREATMENTS (May 20).—These treatments were made when the calyptras of most of the flowers had fallen and the pistils were exposed directly to the growth-regulators. Branches of many treated clusters on May 31 showed much curvature, and some were turning yellow. Many clusters were dead and dried 46 days after treatment, and others were severely damaged (table 1).

Beta-naphthoxypropionic acid and 4-chlorophenoxyacetic acid were the only growth-regulators which resulted in



FIGS. 1, 2.—Clusters of Black Corinth grapes. Fig. 1 (*left*), 85 days after treatment on May 11 with 200 p.p.m. of alpha-naphthaleneacetic acid. Note that rachises are alive and turgid but that ovaries failed to enlarge. Fig. 2 (*right*), treated at flowering with 200 p.p.m. of beta-naphthoxypropionic acid. Many berries failed to enlarge. (Photographed August 4, 1949.)

RESULTS OF FIRST TREATMENTS (May 11).—Two days after treatment the rachis and its branches and the pedicels of many treated clusters showed curvatures. After several weeks some clusters treated with the higher concentrations of growth-regulators had only ovaries to which green calyptras often adhered. By July 5 these calyptras were brown, but many still adhered (fig. 1). At that time many treated clusters were dead. The only responses noted in the clusters treated on May 11 were these injuries. Growth-regulators in aqueous solution generally caused less injury than when in the oil emulsion, and more injury usually resulted from the first than from the second treatment (table 1).

marked responses other than injury. Applications of beta-naphthoxypropionic acid at 200 p.p.m. resulted in larger clusters and berries and hastened maturation. Twelve days after application the framework and berries of treated clusters were noticeably larger than the untreated ones (table 2). By the time of harvest on August 4 the untreated clusters on girdled vines weighed more, however,

TABLE 2

SIZE* OF BLACK CORINTH GRAPE CLUSTERS 12 DAYS AFTER TREATMENT WITH BETA-NAPHTHOXYPROPIONIC ACID

Concentration of acid (p.p.m.)	Length† (mm.)	Diameter of rachis at middle of cluster (mm.)	Diameter of cluster at middle (mm.)	Diameter of berry (mm.)
200 (not girdled)	86	3.3	56	4.3
0 (not girdled)	53	2.0	30	2.8
0 (girdled)	73	2.5	36	3.3

* Average of 6 replicates.

† Length was distance from tip to basal branch or wing.

than the treated clusters on ungirdled vines (table 3). About 60% of the ovaries treated with the compound at 200 p.p.m. did not enlarge (fig. 2), which partially accounts for the relatively small weights of the clusters.

About 20% of the surface of the fruit treated with 200 p.p.m. of beta-naphthoxypropionic acid was blue by July 5, and 1 week later approximately 75% was colored, while the untreated berries were still almost entirely green. On July 19 the surface of berries treated with this compound was almost completely blue, whereas usually not more than 50% of the surface of untreated berries, whether on girdled or ungirdled vines, was blue (fig. 3). The percentage of total soluble solids by weight of the juice of the berries, as measured with a hand refractometer, also indicated that this compound hastened maturity. For example, on July 19 these percentages (average of juice of eight replicate berries) for clusters on ungirdled vines treated with 200 p.p.m. of beta-naphthoxypropionic acid and for untreated berries on ungirdled vines were 23.5 and 12.9, respectively.

Coloration of berries treated with 4-chlorophenoxyacetic acid at a concentration of 50 p.p.m. was much retarded, and these berries were also much larger than those from untreated, ungirdled vines. Berries of clusters treated with 4-chlorophenoxyacetic acid at a concentration of 5 p.p.m. became larger than untreated controls whether the latter were on girdled or ungirdled vines (table 4, fig. 4), but their maturity was not noticeably hastened. About 25% of these berries were very large (about 11 mm. in diameter) and contained seeds or seedlike structures in contrast to the seedless berries of untreated clusters or those receiving other applications. The compound at 0.5 p.p.m. had relatively less influence on size of the berries.

TABLE 3

AVERAGE WEIGHTS (GM.) OF BLACK CORINTH GRAPES TREATED WITH BETA-NAPHTHOXYPROPIONIC ACID. HARVESTED ON AUGUST 4

Concentration of acid (p.p.m.)	Weight per cluster*	Weight of 200 berries†
200 (not girdled)	66	50‡
0 (not girdled)	59	25
0 (girdled)	193	59

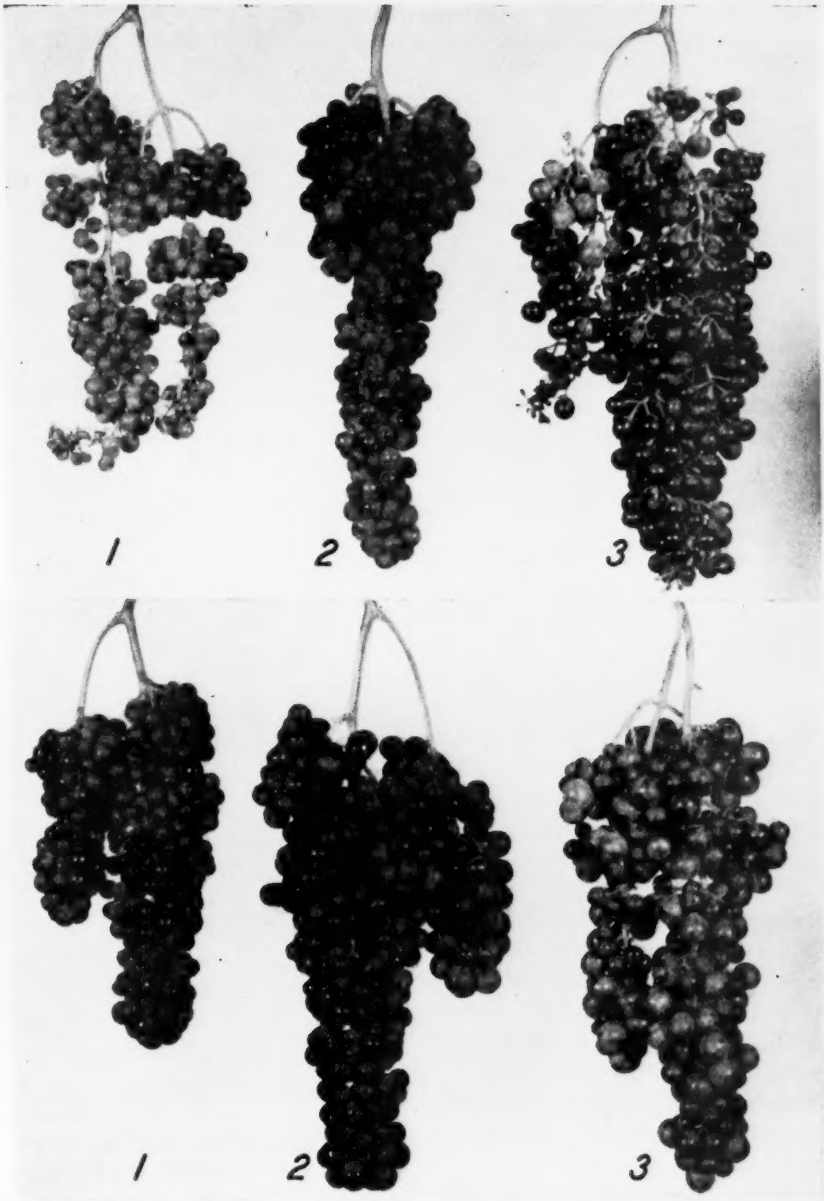
* Six replicates.

† Two replicates.

‡ Ovaries or berries which failed to enlarge were not included.

THOMPSON SEEDLESS

Applications of six growth-regulators in oil emulsions were made on three



FIGS. 3, 4.—Clusters of Black Corinth grapes. Fig. 3 (*upper*), 60 days after date of treatment. *Left to right:* (*1*) untreated, not girdled; (*2*) untreated, girdled; and (*3*) treated with 200 p.p.m. of beta-naphthoxypropionic acid, not girdled. Note that most of berries on treated cluster are darker colored and more mature but that over half of them failed to enlarge. Fig. 4 (*lower*), 85 days after treatment with 5 p.p.m. of 4-chlorophenoxyacetic acid: (*1*) untreated, not girdled; (*2*) untreated, girdled; and (*3*) treated, but not girdled. Note large berries, which contained seeds or seedlike structures, on treated cluster.

dates: June 7, just after the normal drop of berries following flowering had occurred; July 7, when the berries were about three-fourths of their maximum diameter; and on July 28, when the berries were almost maximum size but still quite green. The clusters on half of each of five vines were treated on these dates with beta-naphthoxypropionic acid to observe the response to repeated applications. With the development of the waxy bloom the surface of the berries did not wet thoroughly, so about 0.2% by weight of Dreft was added to the solutions at the last two treatments.

Several days after the treatment on June 7 the rachis and its branches and the pedicels of some clusters showed curvatures (table 1). Some clusters recovered and developed normally, but others were severely injured, with the rachis and its branches and the pedicels thickened, split, and callused. In some cases very few berries enlarged to the size of those on untreated clusters (fig. 5). Some clusters were killed.

The responses were usually successively less from the treatments on July 7 and July 28 (table 1). The gradual shriveling and drying of the berries on some treated clusters were the most striking effects of these treatments.

At harvest on September 13, clusters which had three treatments with beta-naphthoxypropionic acid showed no more injury or other response than those which had received only the one application on June 7.

4-Chlorophenoxyacetic acid was the only compound used that resulted in definite responses other than injury. Pedicels of the clusters treated on June 7 with this compound at a concentration of 200 p.p.m. enlarged to more than twice the thickness of those of untreated controls (fig. 7), but the treated berries were only slightly larger (table 4). The heaviest clusters and berries resulted from the treatment with 4-chlorophenoxyacetic acid at 20 p.p.m. on June 7 (table 4, fig. 6). There was little response to this compound at 2 p.p.m.

TABLE 4

AVERAGE WEIGHTS (GM.) OF BLACK CORINTH AND THOMPSON SEEDLESS GRAPES TREATED WITH 4-CHLOROPHENOXYACETIC ACID. HARVESTED ON AUGUST 4 AND SEPTEMBER 13, RESPECTIVELY

Concentration of acid (p.p.m.)	Weight per cluster	Weight of 200 berries*
	Black Corinth	
50 (not girdled)	52	56
5 " "	103	80†
0.5 " "	67	40
0 " "	59	25
0 (girdled)	193	59
	Thompson Seedless	
200 (not girdled)	617	260
20 " "	836	324
2 " "	454	250
0 " "	472‡	227‡

* Two replicates.

† Two hundred of large seeded berries weighed about 135 gm.

‡ Average of control (untreated) parts of three treated vines.

Discussion

The acceleration of ripening and the enlargement of many of the berries of Black Corinth grapes resulting from application of beta-naphthoxypropionic acid at a concentration of 200 p.p.m. on May 20 was very striking, although many berries failed to enlarge. If treatment had been delayed for several days, it is possible that more of them would have enlarged. The growth-regulators

FIGS, 5, 6, 7.—Untreated and treated clusters of Thompson Seedless grapes 91 days after treatment on June 7. Fig. 5 (*upper*), treated with beta-naphthoxypropionic acid. Untreated (*1*); 200 p.p.m. (*2*) inhibited growth only of berries near apex of cluster, but 1000 p.p.m. (*4*) stunted majority of berries. Intermediate amount of injury resulted from 500 p.p.m. (*3*). Large berries on (*3*) are probably due to fewer number of normal berries per cluster in comparison to untreated, although effects of growth-regulator may be involved. Fig. 6 (*middle*), treated with 20 p.p.m. of 4-chlorophenoxyacetic acid. Note that treated clusters (*3*, *4*) are more compact and have larger berries than controls (*1*, *2*). Fig. 7 (*lower*), treated with 200 p.p.m. of 4-chlorophenoxyacetic acid (*right*). Note thickened pedicels. Control on left.

could not come in contact with the pistils and other parts of those flowers which were covered by the attached calyptras and hence may not have been effective. With a delay in treatment more calyptras would have fallen before application of the compounds. Older clusters probably would have been injured less.

Abscission layers evidently did not develop at the base of many of the calyptras on some of the treated clusters of Black Corinth grapes. These calyptras adhered for several weeks, remaining alive and green.

Applications of 4-chlorophenoxyacetic acid at concentrations of 5 p.p.m. to Black Corinth, although resulting in the largest berries obtained by any of the treatments, would be disadvantageous on account of the seeds or seedlike structures obtained. If these seeds proved to be viable, however, then the growth-regulator might be of value in grape breeding. The development of seeds or seedlike structures resulting from applications of growth-regulator has been reported also in fruits of Navel orange (**10**) and blackberry (**8**).

Young clusters of Thompson Seedless grapes were much more responsive to the growth-regulators than older ones. The clusters treated when berries were quite young on June 7 with concentrations of 20 p.p.m. of 4-chlorophenoxyacetic acid increased markedly in size over the controls. Clusters of both Black Corinth and Thompson Seedless grapes were likewise most severely injured, with few exceptions, at the youngest developmental stages. The clusters which had three treatments with beta-naphthoxypropionic acid showed about the same injury as those which had received only the one application on June 7. Apparently the greatest injury to the re-treated clusters occurred at the first treatment.

The large compact clusters resulting from application of 4-chlorophenoxyacetic acid at a concentration of 20 p.p.m. to Thompson Seedless on June 7 would be undesirable for use as table grapes. Perhaps if the clusters were thinned before or after treatment by removal of several branches or the apical portion, clusters desirable for table grapes might be obtained. WINKLER (**11**) demonstrated that berry thinning of large-clustered varieties of table grapes overcame the tendency of the clusters to be too compact.

Girdling is commonly used in the production of Thompson Seedless grapes for use as table grapes, because it increases the size of the berries (**7**). It would be of interest to study the effects of girdling and thinning of this variety in relation to applications of plant growth-regulators (**7, 11**).

Summary

1. Oil emulsions of beta-naphthoxyacetic acid, alpha-naphthaleneacetic acid, gamma-(indole-3)-*n*-butyric acid, beta-naphthoxypropionic acid, 2,4,5-trichlorophenoxyacetic acid, 4-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, 2-chlorophenoxyacetic acid, alpha-(2-chlorophenoxy)propionic acid, and indole-3-acetic acid were separately applied to Black Corinth grapes by dipping clusters of the flowers at the beginning of or at full bloom. The first three compounds were also applied to Black Corinth in aqueous solution. Oil emulsions of the first six compounds listed were separately applied to Thompson Seedless grapes by dipping clusters of fruit at one of three developmental stages.

2. The treatment at full bloom of flower clusters of Black Corinth with beta-naphthoxypropionic acid at 200 p.p.m. resulted within 12 days in berries

and clusters larger than those of untreated controls. The maturation of these treated clusters was accelerated. Applications of 4-chlorophenoxyacetic acid at 5 p.p.m. resulted in many large berries containing seeds or seedlike structures in contrast to berries of untreated controls or of other treatments which produced smaller seedless fruit. The other compounds resulted in no marked responses other than injury.

3. Very young berry clusters of Thompson Seedless grapes treated with concentrations of 20 p.p.m. of 4-chlorophenoxyacetic acid resulted in larger clusters and berries than untreated controls. The treated clusters were too compact for commercial use as table grapes. Clusters treated with concentrations of 200 p.p.m. of the compound showed little injury but developed pedicels more than twice as thick as the controls. The other compounds produced varying amounts of injury which, in some instances, resulted in the production of large berries, probably owing to the fewer number of normal berries per cluster in comparison with the untreated ones.

4. Young clusters were much more responsive than older ones. Some treatments resulted in only temporary curvatures of the rachis, its branches, and the pedicels; in more severe treatments the rachis and its branches enlarged, split, and callused, and many berries failed to enlarge; some clusters were killed.

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RECOVERY AND VIABILITY OF MESQUITE SEEDS FED TO SHEEP RECEIVING 2,4-D IN DRINKING WATER[1]

G. E. GLENDENING AND H. A. PAULSEN[2]

Introduction

Mesquites (*Prosopis* spp.) are leguminous trees or shrubs which occur naturally in the warmer parts of the United States. They range from southern Kansas to southeastern California. The increase in abundance of these plants, on semidesert and shortgrass ranges during the last 75–80 years, has resulted in a marked decrease in forage production of perennial range grasses and is of major economic concern. Mesquites, in stands of varying density, are now present on nearly 70 million acres of range land within the states of Arizona, New Mexico, and Texas. In Texas alone the unwanted presence of mesquite has been reported to be causing an economic loss amounting to some 50 million dollars annually (8). In southern Arizona stands of velvet mesquite (*P. juliflora* var. *velutina* [Woot.] Sarg.) are increasing in density; and, as a result of invasion by these xerophytic plants, much grassland is being rapidly converted to a woodland type of lower value for livestock use.

Mesquite seeds are borne in fleshy pods which are eaten greedily by cattle, horses, and sheep. Many seeds pass through the animals as whole, undigested seeds. The exact significance of this fact in the spread of mesquite is not well understood. Since velvet mesquite reproduces entirely from seed, however, it is probable that a significant reduction in both spread and abundance of this plant on range lands could be accomplished if a practical method were found to prevent the germination or development of seedlings from seeds disseminated in the droppings of domestic animals.

FISHER (3) has shown that 54, 45, and 12% of the seeds of honey mesquite (*P. juliflora* var. *glandulosa* [Torr.] Cockerell) recovered from droppings of lambs, calves, and mules, respectively, were viable. Data on rate of elimination of the seeds and the relative amount of seed destroyed by the digestion processes were not presented.

ROSENFELS (9) reported on the spread of white-top (*Hymenophysa pubescens* C. A. Mey.) seed in the droppings of grazing cattle on range land in Nevada. He obtained no data on time required for elimination of the seeds but suggested that viability of the seeds may be enhanced by digestive processes.

BURTON and ANDREWS (2), who studied the rate of elimination of seeds of lespedeza and various species of forage grasses, found that, although the bulk of the seeds are passed in 72 hours, the time required varied from 24 hours to 10 days depending on the type of seeds eaten.

[1] This study is a part of investigations being conducted by the Southwestern Forest and Range Experiment Station in co-operation with the University of Arizona Agricultural Experiment Station under RMA Project RM:b-4 for research on the basic ecology and physiology in control of undesirable range plants. Valuable assistance in conducting this study was rendered by E. B. STANLEY and MAX ROBINSON, Professor and Assistant Professor, respectively, Animal Husbandry Department, University of Arizona.

[2] Southwestern Forest and Range Experiment Station. Maintained by the Forest Service, U.S. Department of Agriculture for Arizona, New Mexico, and West Texas, with headquarters at Tucson, Arizona.

HAMNER *et al.* (**5, 6**) have reported on the devitalizing effect of 2,4-dichlorophenoxyacetic acid (2,4-D) at concentrations as low as 1–10 p.p.m. upon seeds of various weeds and on peas and beans. Other reports (**1, 7**) have shown that 2,4-D in low concentrations is harmless to warm-blooded mammals.

The study reported here was made in connection with other investigations of the reproduction of velvet mesquite and was aimed at determining the extent to which germination of mesquite seed and growth of resulting seedlings might be affected by exposure to 2,4-D introduced into the digestive tract of sheep. Data on the rate of passage through the alimentary tract and the ratio of seeds eaten to seeds recovered are also reported. The experiment was conducted at the feeding barns of the Arizona Agricultural Experiment Station at Tucson, Arizona, during October and November, 1948. So far as is known, no previous studies have been reported on the effects of direct feeding of 2,4-D upon seeds of a noxious plant fed concurrently to domestic animals.

Experimental methods

Two sound-mouthed Rambouillet ewes were taken off pasture and confined for 5 days in small, cement-floored stalls before the feeding study began. On the following day and thereafter, at 7-day intervals, each sheep was given, in addition to its daily ration of alfalfa hay, approximately 75 gm. of mesquite pods broken into small segments and containing a total of 500 seeds. Each morning for 7 days following the feeding of the seeds, all fecal pellets eliminated during the previous 24-hour period were collected. The pellets were then broken up mechanically and screened for mesquite seeds. Daily records were maintained separately for each sheep.

The seed feeding and recovery procedure was repeated for six feeding periods during the final three of which 2,4-D was added to the drinking water in the free acid form at a concentration of 100 p.p.m. The water was accessible to the sheep at all times and was replenished with water containing 2,4-D as it was consumed; approximately 1 gallon per day was consumed by each sheep.

Immediate and total potential viability of the seeds recovered from the pellets was determined by germination at approximately 78° F. on moist blotting paper in Petri dishes. Seeds were removed individually when germination occurred. Those seeds which had not germinated after 19 days were mechanically scarified, and the tests were continued for an additional 4 days or until positive germination had occurred. Emergence of the radicle to a distance of 2 mm. was interpreted as positive germination.

Results

ELIMINATION OF SEEDS.—Some variation occurred in the daily and total elimination of seeds by the two sheep and between comparable feeding periods; but, since tests showed that these differences were not statistically significant, the data for the two sheep were combined (table 1). Of a total of 5950 mesquite seeds eaten during the six feeding periods, 1899, or 31.9%, were recovered as whole seeds. In addition, a considerable number of seed fragments were identifiable in the fecal material. The slightly greater number of seeds recovered during those feedings when 2,4-D was present in the drinking water is not statistically reliable. The data indicate a marked curvilinear relationship between elapsed time following ingestion and elimination of mesquite beans in fecal pellets (fig. 1).

TABLE 1

DAILY ELIMINATION AND VIABILITY OF MESQUITE SEEDS FED TO SHEEP*

No. of days after feeding	No. of whole seeds recovered		% viability when recovered		% viability after scarification	
	Without 2,4-D	With 2,4-D	Without 2,4-D	With 2,4-D	Without 2,4-D	With 2,4-D
1	70	72	1.4	8.3	62.9	50.0
2	223	491	3.6	0.8	69.1	82.3
3	256	267	1.6	2.2	89.5	85.8
4	148	66	4.0	0.0	95.9	97.0
5	112	91	0.9	1.1	89.2	92.3
6	44	27	2.2	7.4	100.0	88.9
7	11	16	0.0	0.0	100.0	87.5
8	5	.....	0.0	.....	100.0	.....
Total	869	1030	2.42	1.84	83.9	83.0

* Includes all seeds recovered from two sheep during three feeding periods.

VIABILITY OF RECOVERED SEEDS.—The viability of check seeds from the same lot as those fed, based on three tests of 100 seeds each, was 6% prior to, and 94% after, scarification of the seed coats. Only 2.42 and 1.84%, respectively, of the seeds recovered during feeding periods when normal drinking water and water treated with 2,4-D were available to the sheep were immediately viable when removed from the pellets and germinated on moist blotting paper. Following scarification of seed coats by nicking, viability of these seeds was 83.9 and 83.0%, respectively. These comparisons suggest that most of the seeds which have permeable seed coats, and which thus might be acted upon by the 2,4-D, are destroyed by digestive processes.

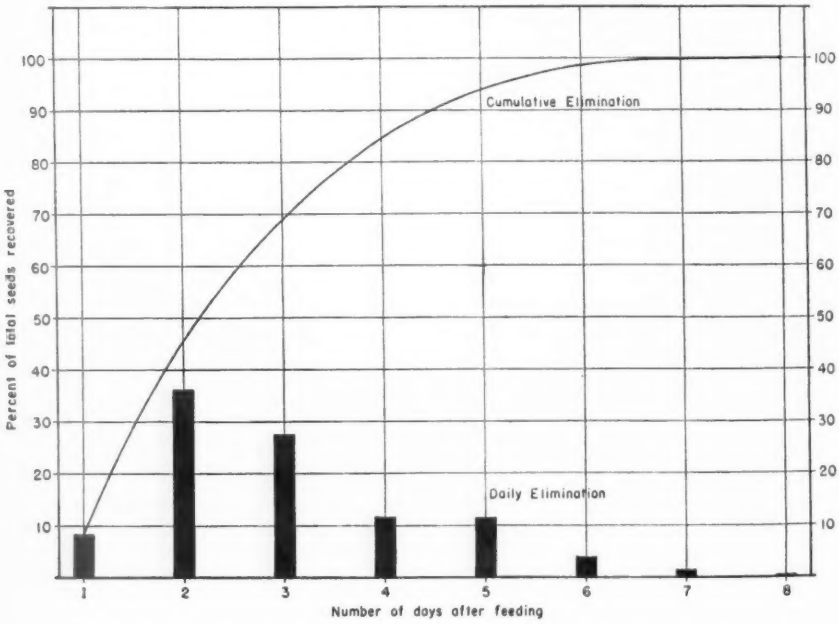

FIG. 1.—Average daily and cumulative elimination of mesquite seeds based on average obtained from two sheep during six separate feeding periods.

DISPOSITION OF SEEDS EATEN.—Data so far shown are based only on those seeds which were recovered intact from fecal pellets. These represent less than one-third (31.92%) of the total seeds fed to the sheep and do not account for the bulk of the seeds eaten. A summary of the critical data based on the total number of seeds eaten is given in table 2.

Although less than 1% of the total seeds eaten were eliminated in an immediately germinable condition, more than one-fourth (27.29%) contained viable embryos and were capable of germination if scarified. A considerable number of the recovered seeds (14.4%) were blackened and partially swollen, indicating that they had been acted upon by the digestive processes. These blackened seeds did not germinate and were discarded from the germination tests after an average period of 8 days because of excessive mold growth and fermentation. The remainder of the recovered whole seeds were apparently unchanged except for the removal of the sugary pericarp.

TESTS FOR 2,4-D IN FECAL PELLETS.—Chemical tests made with chromotropic acid according to a method described by FREED (4) gave a strong positive indication that the fecal pellets contained 2,4-D.[3] Evidence that the 2,4-D was present in the fecal pellets in phytotoxic concentrations was obtained as follows: (*a*) a marked inhibiting effect was observed on mesquite seedlings when pregerminated for 24 and 48 hours, placed in the fecal material, and cultured on moist sand. Measurements of radicles and hypocotyls showed that the degree of inhibition was similar to that obtained when comparable seedlings were cultured in Petri dishes containing solutions of 2,4-D at a concentration of 50 p.p.m.; (*b*) scarified mesquite seeds, placed in pellets collected prior to the addition of 2,4-D to the drinking water, germinated normally, but those placed in pellets collected after the feeding of the growth inhibitor did not germinate.

Subsequent tests showed that detectable quantities of 2,4-D still remained in the fecal pellets when tested after 60 and 150 days' exposure to the atmosphere in the greenhouse, suggesting that some additional seeds may be destroyed before the 2,4-D is dissipated from the pellets (fig. 2).

TABLE 2*

EFFECTS OF MASTICATION AND DIGESTION BY SHEEP ON MESQUITE BEANS

	Number	%
Total seeds eaten	5950	.....
Seeds germinable when eliminated	40	0.67
Seeds germinable when seed coat was nicked	1584	26.62
Total seeds eliminated in viable condition	1624	27.29
Seeds eliminated intact but nonviable	274	4.60
Total intact seeds eliminated	1899	31.92
Seeds digested or eliminated as fragments	4051	68.08

* All figures represent combined data from two sheep.

There was no physical evidence of any detrimental effects on the experimental sheep as a result of the daily consumption of approximately 1 gallon of water containing 0.36 gm. of 2,4-D acid for a period of 23 consecutive days.

Summary

1. Of a total of 5950 velvet mesquite seeds which were fed to two sheep during six separate feeding periods, 68% were destroyed beyond recognition by mastication and other digestion processes.

[3] These tests were made by BYRON O. BLAIR, plant physiologist, Southwestern Forest and Range Experiment Station.

Greatest elimination of seeds occurred the second day after ingestion, and approximately 95% of the seeds were eliminated by the fifth day. That some seeds require at least 8 days to pass through the alimentary tract, coupled with the amount of travel of the livestock which may occur during such a period, clearly indicates the manner in which livestock may be an important factor in the spread of mesquite on range lands.

4. The addition of 2,4-dichlorophenoxyacetic acid (2,4-D) to the sheep's drinking water at a concentration of 100 p.p.m. resulted in the accumulation of phytotoxic amounts of this substance in the manure. The presence of 2,4-D had only a slight effect on total germination the seed coat being 2.42 and 93.9%, respectively, as compared with 6.0% and 94% for unfed check seeds cultured under comparable conditions.

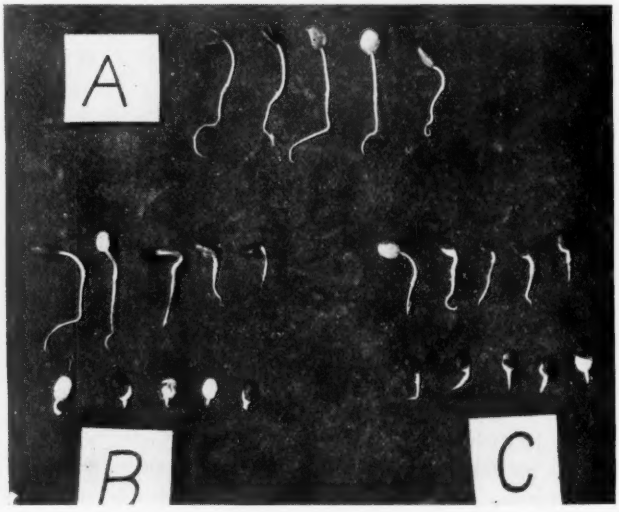

FIG. 2.—Inhibition of mesquite seedlings by 2,4-D in fecal pellets 150 days after elimination. All seedlings shown were 72 hours old and were grown from scarified seed placed on moist sand in Petri dishes. *A*, seeds placed on surface of sand without fecal material. *B*, seeds placed in hole drilled in normal fecal pellet (*top*) and pellet from sheep fed, 2,4-D (*bottom*). *C*, seeds placed on surface of sand and covered with pulverized material from normal fecal pellet (*top*) and pellet from sheep fed 2,4-D (*bottom*).

2. During periods when mesquite is in fruit, movement of livestock directly from an area infested with mesquite to areas not infested would seem unwise.

3. Of the total seeds fed, 27.3% were found to be immediately or potentially viable after passage through the sheep. Germination of those seeds recovered as intact seeds was slightly reduced by passage through sheep, with the average germination prior to and after nicking of the eliminated seeds, because the bulk of the viable seeds have an impermeable seed coat even after passage through the alimentary tract. Seeds which are in a suitable condition for the absorption of water when eliminated by the sheep are inhibited by the presence of the 2,4-D and do not germinate in the pellet. Phytotoxic amounts of 2,4-D are retained in dry pellets for at least 150 days under greenhouse conditions.

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SIX NEW PLANT-GROWTH-INHIBITING COMPOUNDS

J. W. WIRWILLE[1] AND J. W. MITCHELL[2]

The following ammonium compounds[3] have been found to inhibit the growth of snap-bean plants under greenhouse conditions without causing malformation of leaves, stems, roots, or flowers:

(4-Hydroxy-5-isopropyl-2-methylphenyl)trimethyl ammonium chloride, 1-piperidinecarboxylate
(4-Hydroxy-5-isopropyl-2-methylphenyl)trimethyl ammonium iodide, *N*-β-chloroethyl-*N*-ethylcarbamate
(4-Hydroxy-5-isopropyl-2-methylphenyl)trimethyl ammonium iodide, 1-piperidinecarboxylate
(4-Hydroxy-2-isopropyl-5-methylphenyl)trimethyl ammonium iodide, 4-morpholinecarboxylate
(4-Hydroxy-2-isopropyl-5-methylphenyl)trimethyl ammonium chloride, 4-morpholinecarboxylate
(4-Hydroxy-5-isopropyl-2-methylphenyl)trimethyl ammonium chloride, *N*, *N*-bis-(β-chloroethyl) carbamate

These compounds are solids, soluble in water. They inhibited the growth of young bean plants when applied as aqueous sprays and also when applied in a paste made of lanolin containing Tween-20. When applied in a paste at one dosage level (approximately 0.1 mg/plant) to stems of Black Valentine snap-bean seedlings, the amount that the respective compounds reduced stem elongation varied between 44 and 51% as measured 10 days after application.

(4-Hydroxy-5-isopropyl-2-methylphenyl)trimethyl ammonium chloride, 1-piperidinecarboxylate (Amo-1618), one of the more active of the compounds, inhibited stem elongation of bean plants to a greater extent than did 2,4-dichlorobenzylnicotinium chloride, the most active nicotinium compound previously reported (2) and was also more effective than maleic hydrazide (3). The relative

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[3] Supplied by the National Research Council, Chemical Biological Coordination Center: originally synthesized by Dr. R. L. SHRINER, Iowa State College.

activity of these three unrelated compounds was compared at one dosage level (approximately 0.1 mg/plant). Amo-1618 was applied in a paste carrier as a narrow band around the stem of each plant in one group, midway between the first and second nodes. The paste consisted of 16.75% Tween-20, 82.25% lanolin, and 1% of the chemical being tested. A similar group of plants was treated with maleic hydrazide, a third with 2,4-dichlorobenzylnicotinium chloride, and a fourth group was left untreated. Eight days after treatment, average stem elongation was reduced 47, 39, and 34% by the Amo-1618, maleic hydrazide, and 2,4-dichlorobenzylnicotinium chloride, respectively, in comparison with the controls.

In an experiment in which leaves were treated, Amo-1618 reduced stem elongation of bean seedlings by an average of 24% during an 8-day period following application. Approximately 0.1 mg. of the compound in lanolin–Tween-20 paste was applied to the upper surface of one of the primary leaves of each plant. Neither maleic hydrazide nor 2,4-dichlorobenzylnicotinium chloride reduced stem elongation when applied to primary leaves of comparable bean plants in a similar manner.

Bean seedlings were relatively sensitive to application of Amo-1618 to the leaves. A 0.01-ml. droplet of water containing 100 μgm. of the compound and 2% of Tween-20 was applied to the upper surface of one primary leaf of each plant in one group. The area treated was limited to approximately 0.5 sq. cm. by means of a thin lanolin ring stamped on the upper surface of each leaf just prior to application. An equal amount of maleic hydrazide was applied in a similar manner to another group of seedlings, and a third group was left untreated. Ten days later the average stem length of plants treated with the ammonium compound was 32% less than that of the untreated ones. In contrast, stems of plants treated with maleic hydrazide were 8% longer than stems of untreated ones.

Response of bean seedlings to applications of Amo-1618 to their stems was qualitatively different from that induced by maleic hydrazide. In one experiment approximately 0.1 mg. of each chemical in lanolin–Tween-20 carrier was applied separately to the stems. After prolonged treatment (2 weeks on the first internode), maleic hydrazide brought about abscission of the terminal buds. Such abscission was not induced by either Amo-1618 or 2,4-dichlorobenzylnicotinium chloride.

In general, effects of the ammonium compounds on snap beans have been to inhibit internodal elongation and growth of the trifoliolate leaves without noticeably distorting the shape of the leaves or causing malformation of the stems. Thickness of the leaves appeared to be increased on treated plants, and these plants also developed a deeper green color than did untreated ones. Approximately 0.1 mg. of Amo-1618 applied in a lanolin–Tween-20 paste to the stems for a period of 8 days brought about a 17% increase in dry weight of the primary leaves of the seedlings. It caused only 5% reduction in dry weight of stems, 75% reduction in dry weight of trifoliolate leaves, and had no effect on dry weight of roots. In color and general appearance roots of treated plants were not visibly different from those of untreated ones. The total amount of solid matter in treated plants was 11% less than that of the controls at the end of the treatment period.

TABLE 1

EFFECT OF AMMONIUM COMPOUNDS ON HEIGHT OF BEAN PLANTS, COMPARED WITH CONTROLS. APPROXIMATELY 0.1 MG. OF CHEMICAL IN LANOLIN–TWEEN-20 CARRIER* WAS APPLIED AS NARROW BAND AROUND FIRST INTERNODE OF RESPECTIVE BEAN SEEDLINGS. PERIOD OF TREATMENT, 1 MONTH

Compound	Percentage reduction in stem elongation
(4-Hydroxy-5-isopropyl-2-methylphenyl)trimethyl ammonium chloride, 1-piperidinecarboxylate	67
(4-Hydroxy-5-isopropyl-2-methylphenyl)trimethyl ammonium iodide, *N*-β-chloroethyl-*N*-ethylcarbamate	59
(4-Hydroxy-5-isopropyl-2-methylphenyl)trimethyl ammonium chloride, *N*, *N*-bis-(β-chloroethyl) carbamate	53
(4-Hydroxy-5-isopropyl-2-methylphenyl)trimethyl ammonium iodide, 1-piperidinecarboxylate	50
(4-Hydroxy-2-isopropyl-5-methylphenyl)trimethyl ammonium iodide, 4-morpholinecarboxylate	49
(4-Hydroxy-2-isopropyl-5-methylphenyl)trimethyl ammonium chloride, 4-morpholinecarboxylate	47

* Paste contained 1% of substance tested, 16.75% Tween-20, and 82.25% lanolin.

FIG. 1.—Effect of (4-hydroxy-5-isopropyl-2-methylphenyl)trimethyl ammonium chloride, 1-piperidinecarboxylate on growth of Black Valentine snap-bean plants. *Left*, untreated; *right*, approximately 0.1 mg. of compound applied to first internode when plant was in seedling stage (3 days after emergence). Photographed 1 month after treatment. Note pods on untreated plant; treated plant had not blossomed at time photograph was taken.

Inhibiting effects of the ammonium compounds persisted in bean plants grown to maturity under greenhouse conditions. After flowering and at a time when vegetative growth of the control plants had practically ceased, the latter were three times as tall as others whose stems had been treated with approximately 0.1 mg. of Amo-1618 when in the seedling stage (table 1).

The blooming date of Black Valentine bean plants was delayed 3–10 days when approximately 0.1 mg. of each of the various ammonium compounds in lanolin–Tween-20 paste was applied separately to the stems of seedlings. Amo-1618 was the most effective compound in this respect (fig. 1). Flowers on treated plants developed a somewhat deeper color than did flowers on untreated ones. There was no marked difference in the average number of flowers finally produced under the conditions used. Observations on flowers were made in two experiments, with ten plants per treatment. No attempt was made to evaluate slight effects on flower number which might have proved significant had larger

numbers of plants been involved and the results evaluated statistically.

In general, the effects of aqueous sprays containing the ammonium compounds were similar to effects obtained by use of pastes containing the substances. Aqueous-spray solutions containing from 500 to 1500 p.p.m. of Amo-1618 and 0.5% Tween-20 inhibited stem elongation of bean seedlings and checked the development of trifoliolate leaves without causing apparent malformation. A similar spray containing 4500 p.p.m. injured the plants by checking expansion of the primary leaves and causing them to become wrinkled near the margins. The inhibiting effect of this concentrated spray on internodal elongation and on development of trifoliolate leaves was very marked.

The ammonium compounds were most effective when applied to relatively young, rapidly growing plants. This is known to be true of other growth-regulators, such as 2,4-dichlorobenzylnicotinium chloride and maleic hydrazide (1, 2). Lanolin–Tween-20 paste containing approximately 0.1 mg. of Amo-1618 greatly inhibited stem elongation and trifoliolate leaf growth of bean plants when applied to their stems 2 days after the seedlings emerged. Applied to other plants in the same group 8 days later, the chemical caused the stems to elongate less than the controls and to become very thick and sturdy; but the effect on both stems and leaves was much less pronounced than when the plants were treated during an earlier stage in their development.

Amo-1618 did not visibly affect the growth of plants of tomato, cabbage, cauliflower, and eggplant when applied to the young seedlings.

Care should be exercised in experimenting with the ammonium compounds described here until more is known about their effects on animals and man.

Summary

1. Six organic ammonium compounds were found to inhibit internodal elongation of Black Valentine bean seedlings when applied to the stems or leaves:

(4-Hydroxy-5-isopropyl-2-methylphenyl)trimethyl ammonium chloride, 1-piperidinecarboxylate
(4-Hydroxy-5-isopropyl-2-methylphenyl)trimethyl ammonium iodide, *N*-β-chloroethyl-*N*-ethylcarbamate
(4-Hydroxy-5-isopropyl-2-methylphenyl)trimethyl ammonium iodide, 1-piperidinecarboxylate
(4-Hydroxy-2-isopropyl-5-methylphenyl)trimethyl ammonium iodide, 4-morpholinecarboxylate
(4-Hydroxy-2-isopropyl-5-methylphenyl)trimethyl ammonium chloride, 4-morpholinecarboxylate
(4-Hydroxy-5-isopropyl-2-methylphenyl)trimethyl ammonium chloride, *N*, *N*-bis-(β-chloroethyl) carbamate

2. These ammonium compounds delayed blossoming of bean plants 3–10 days when applied in amounts of approximately 0.1 mg. per plant.

3. One of the more active of these compounds, (4-hydroxy-5-isopropyl-2-methylphenyl)trimethyl ammonium chloride, 1-piperidinecarboxylate, proved to be more effective in inhibiting stem elongation of bean seedlings than did either maleic hydrazide or 2,4-dichlorobenzylnicotinium chloride.

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